Surveillance of vector-borne diseases in cattle with special emphasis on bluetongue disease in Switzerland

INAUGURALDISSERTATION

zur

Erlangung der Würde einer Doktorin der Philosophie

vorgelegt der

Philosophisch-Naturwissenschaftlichen Fakultät der

Universität Basel

von

Vanessa Nadine Racloz Bouças da Silva

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Genehmigt von der Philosophisch-Naturwissenschaftlichen Fakultät

Der Universität Basel auf Antrag von

Prof. Dr. Marcel Tanner, P.D. Dr. Christian Griot und Prof. Dr. Katharina Stärk,

Basel, den 8. Februar 2008

Prof. Dr. Hans-Peter Hauri
Dekan

dedicated to my family-

Jacques, Helga, Amaro and Alberto

Acknowledgments

First and foremost, I would like to thank my supervisor Prof. Katharina Stärk for her expert guidance and continuous support throughout my PhD, especially for knowing how to nudge me into action. I would also like to thank both her and PD Dr. Christian Griot for their feedback, encouragements and discussions which have helped me enormously.

I would also like to thank everyone in the monitoring team and at BVET, who one way or the other have assisted and provided me with advice be it technical or supportive. Special thanks go to Dr. Barbara Häsler, Dr. Cornelia Neff, and Dr. Sonia Menendez who helped me tackle the cows, as well as Dr. Simona Casati and Monika Kuhn in fighting through all those bugs. My thanks also goes to Michelle Vock for answering my unending stream of questions. Likewise, I would like to thank Dr. Patrick Presi and Dr. Heinzpeter Schwermer for their critical approach to my work and support in GIS matters, and to Dr. Penelope Vounatsou for helping me plough through the statistics. I would also like to show my appreciation to Dr. Martin Reist, Dr. Eric Breidenbach and Dr. Daniela Hadorn for their fruitful collaborations.

A big thanks also to those who have provided me with impromptu advice and tips and to all the new people I have met through conferences and meetings for making me always feel welcome.

I would like to thank Dr. Barbara Thür and Dr. Ariane Cagienard for their help, as well as colleagues from the Institute of Parasitology in Zürich, the Swiss Tropical Insitute in Basel, and the Center for Zoonoses, Bacterial animal diseases and Antibiotic resistance (ZOBA) in Bern.

Furthermore, I would like to thank Prof Katharina Stärk, PD Dr. Christian Griot, Dr. Francois Roger and Prof. Marcel Tanner, the members of my PhD committee for their interest and confidence in this study.

Many thanks to all my friends, whose laughter and entertainment have been great throughout the continents and years.

And last but not least, my deepest appreciation goes to my parents Jacques and Helga as well as my little brother Amaro and my husband Alberto, for their trust, humour, love, encouragement and faith in me, of which without I would not be who I am today.

Summary

Due to previous climatic conditions in Switzerland, vector-borne diseases were not of primary importance to the Swiss Federal Veterinary Office. It has now been established that global warming has had a major impact on vector species ecology due to temperature shifts, humidity and precipitation changes amongst many factors, which influence vector habitats and their distribution, and allowed in some instances for vectors already present in an area to transmit a disease, or aid in the extension of habitats of exotic vectors species.

In Switzerland, several vector-borne diseases have and are emerging, causing alarm due to the eventual consequences in health and economic matters that they can bring with them. Such examples are Bluetongue disease (BT), cattle anaplasmosis and cattle babesiosis, which have, at the time of writing, now all been reported in Switzerland. In order to aid decision and policy makers in planning eventual surveillance, prevention and control measures, disease surveillance needs to be focused on aspects of vector ecology and the epidemiology of the mentioned diseases.

In this study, surveillance took form as a sentinel herd strategy through serological and entomological sampling over the past three years. The aim was to establish an early warning system for the primary incursion of BT virus via infected *Culicoides* species vectors, or the re-emergence of anaplasmosis and babesiosis through endemic tick species. Hence, as a risk based approach, it was important to identify areas considered at higher likelihood of disease occurrence located within Switzerland. This was achieved through the collection of climatic, environmental, altitude, entomological and vector population dynamics data. These data were incorporated into a Geographic Information System and a mathematical model and finally developed into a Scenario Tree pathway to help decide upon different surveillance system components.

Models developed in this study through the collected data have proved useful as the first cases of reported BT disease in Switzerland were in areas highlighted by the suitability maps, as well as mirroring calculated prevalence estimates. Results from this study were also implemented into a national surveillance plan for bluetongue disease in Switzerland.

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Abbreviations

BT	Bluetongue
BTV	Bluetongue virus
CAT	Card Agglutination Test
CDC	Centers for Disease Control and Prevention
ELISA	Enzyme Linked Immunosorbent Assay
ESRI	Environmental Systems Research Institute
GIS	Geographic Information System
IAH	Institute of Animal Health
IFAT	Indirect Immunofluorescence Antibody Test
IPZ	Institute of Parasitology
IVI	Institute of Virology and Immunoprophylaxis
OIE	World Animal Health Organisation
PCR	Polymerase Chain Reaction
SFVO	Swiss Federal Veterinary Office
SSC	Surveillance System Component
STI	Swiss Tropical Institute
WHO	World Health Organisation
ZOBA	Center for zoonoses, bacterial animal diseases and antibiotic resistance

CHAPTER 1

Introduction

1.1 Overview of vector borne diseases on a global scale

Created in 1924, the World Organization for Animal Health (OIE) was set up with the primary aim of fighting animal diseases on a global level. This organization is responsible for improving animal health on a worldwide basis through the collection, analysis and dissemination of scientific information important to the veterinary community. Among its information distribution system is the 'OIE notifiable list' (previously known as lists A and B) regarded as containing a list of diseases which affect animal health in a consequential manner, affecting socioeconomic and/or public and veterinary health matters and are significant in the international trade of animals and animal products. Of the 66 listed diseases affecting the categories of bovine, swine, caprine, ovine, equine and multi species disease, 23 are considered as vector-borne diseases (Table 1.1) (OIE). Vector-borne diseases are described as a disease which is transmitted from one infected individual to another through an arthropod, or agent serving as an intermediary host (Center for Disease Control and Prevention, www.cdc.gov/ncidod/dvbid). This transmission is dependent on three stages involving the infectious agent, the vector and finally the host(s).

The diseases which appear on the OIE list have been chosen for two main reasons: the economic impact caused by an outbreak and its consequences, as well as the veterinary health factor. One of these diseases which causes both large financial and social damages to an affected region, as well as being able to provoke serious morbidity and mortality rates is the arboviral disease (arthropod-borne viral disease) called "bluetongue" (BT). Belonging to the group of arthropod-borne diseases, bluetongue was included as an A listed disease in the previous OIE list, although its status as a serious animal disease has been questioned due to the negative impact on trade and animal movement that is imposed during an outbreak (Gibbs and Greiner, 1994). Apart

from being endemic in many tropical and subtropical regions, BT started to be seen as a threat in the western countries mainly due to large outbreaks in Cyprus in 1943, which presented a mortality rate of 60-70% (Polydorou, 1978) and Portugal and Spain in 1956, which caused the deaths of circa 180,000 sheep in the first few months of the outbreaks (www.fao.org).

Factors affecting vector-borne disease spread

Along with BT, several other vector-borne diseases have been emerging into areas for the first time or previously eradicated especially in the last half century, including many zoonoses such as malaria, dengue fever, shistosomiasis and the plague (Watson *et al.*, 2005). This has been attributed to a range of factors either climate driven ones or associated to changes in industry and urbanization. These can all affect the spread of vector-borne diseases by increasing the ability of the vector to transmit a disease, or in creating suitable habitats for it to establish. In some scenarios, whether accidental or induced by man made phenomenon, diseases which had been related to one type of vector species have also been seen in new vectors which might have different hosts and habitats, as is the case for West Nile disease (Gubler, 2007). Human travel, increased transhumance, deforestation, irrigation projects and the upsizing of farm animal holdings are a few examples of factors associated to vector-borne disease spread created artificially, whilst temperature changes, El Niño activities and general global warming belong to climate driven factors responsible for this occurrence (Gratz, 1999). As described in the World Health Organization work group on climate change (www.who.int/globalchange/climate/), the habitats of certain vectors are indirectly being expanded due to larger areas and altitudes becoming warmer as well as extreme climatic events bringing abnormal quantities of rainwater or heat waves, each affecting the transmission of vector-borne diseases in specific ways.

Table 1.1: Vector-borne diseases in the OIE notification list and their geographic distribution. (www.oie.int, www.who.int,) Arranged by disease type : virus, bacteria and parasite

Vector-borne disease	Occurrence	Vector	Hosts
African horse sickness	Africa, Middle East, Europe	Culicoides imicola and C. bolitinos	Equine
African swine fever	Sub-Saharan Africa, Europe	Ornithodoros tick species	Swine
Bluetongue	Worldwide	*Culicoides midge species*	Ruminants
Crimean Congo- hemorrhagic fever	Africa, Europe, Balkans, South Africa and Asia	*Argasid or ixodid ticks*	wild and domestic animals (zoonosis)
Equine encephalomyelitis (Eastern and Western)	Canada, Caribbean, North, South and Central America	Mosquito species	Equine, birds (zoonosis)
Equine infectious anaemia	Worldwide	Biting flies, mosquitoes	Equine
Japanese encephalitis	Asia, Australia, India	*Culex* mosquito species	Pigs, birds *(zoonosis)*
Lumpky skin disease	Africa, South and North Africa, Isreal	Arthropods	Cattle, zebus, giraffe, impala
Nairobi sheep disease	East Africa	*Rhipicephalus* ticks	Sheep and Goats
Rift Valley fever	Africa	*Mosquito species*	Multiple species (zoonosis)
Venezuelan equine encephalomyelitis	North, Central and South America	Mosquito species	Equine (zoonosis)
Vesicular stomatitis	United States, Europe, South Africa	*Arthropods*	Mammals (zoonosis)
West Nile fever	Worldwide	*Mosquito species*	Multiple species (zoonosis)
Bovine anaplasmosis	Worldwide	Tick species	Cattle
Bovine babesiosis	Worldwide	Tick species	Cattle
Tularemia	North America, Europe and Asia.	Arthropods	Rodents, rabbits, hares
Equine piroplasmosis	Worldwide	*Ixodid ticks*	Equine
Heartwater	Africa, West Indies	*Amblyomma* tick species	Ruminants
Leishmaniosis	Worldwide	*Phlebotomus* and *Lutzomyia* sandflies	Dogs, rodents, opossums *(zoonosis)*
Surra (Trypanosoma evansi)	Asia, Africa and South America	*Tabanus species (biting flies)*	Multiple species
Theileriosis	Worldwide	Ixodid ticks	Bovidae and ruminants
Trypanosomosis	Africa, South and Central America	*Glossina species (tse-tse)*	Cattle (zoonosis)

Relevance of vector-borne diseases in Switzerland

Until the last century, as stated in (Kalluri et al., 2007), the main cause of human deaths were due to vector-borne transmitted diseases, and the application of hygienic measures, drugs, surveillance systems and vector control caused the disappearance of many of these diseases from Europe. Yet in the last decade, through the impact of natural disasters and climate change, these diseases remain an important burden to both public and veterinary health due to the re-emergence and spread of many vector-borne diseases. According to the Intergovernmental Panel on Climate Change (IPCC, www.ipcc-wg2.org) and the Federal Office for the Environment (FOEN, www.bafu.admin.ch), the increased occurrence of flooding, heat waves and higher altitude limits for snowfall, is likely in most of Europe and more importantly in Austria and Switzerland. These events will directly affect the biology of vectors and consequently the transmission rates of the diseases they cause. In the Swiss economy, cattle and milk products still create around 3 billion Swiss francs per year (www.bfs.admin.ch), hence any diseases affecting this market will have serious implications for farmers and stakeholders. Prior to October 2007, Switzerland had a single serious outbreak of tick-borne diseases in the eastern part of the country (Hofmann-Lehmann *et al.*, 2004) which incited the need to address vector-borne diseases in a new light. Additionally, a study on bluetongue vectors in Switzerland (Cagienard et al., 2004), demonstrated the abundant presence of BT vectors, and combined with the circulation of several BT serotypes in neighboring countries, prompted the study behind this thesis.

1.2 Epidemiology of vector-borne diseases relevant to this project

Bluetongue disease

Bluetongue is a vector-borne, non-contagious, viral disease which affects domestic and wild ruminants (www.oie.int). The virus, of which there are currently 24 known serotypes, is a member of the Reoviridae family and Orbivirus genus. Although distributed worldwide, many serotypes are linked to a specific BT vector, and have been usually restricted to certain geographic regions. For example BTV -1, -3, -9, -15, -16, -20, -21 and -23 occurring in Northern Australia, BTV -1, -3, -4, -6, -8, -12 and -17 circulating in the Central American-Caribbean

Basin, and BTV1–16, -18,-19 and -24 being responsible for African outbreaks (www.iah.bbsrc.ac.uk).

Previous to 2006, BT had been circulating in the Mediterranean and Balkan regions since the late 1990s on a regular basis (Purse *et al.*, 2006), with the exception of the sporadic outbreaks in the 50's mentioned above, causing great economic losses mostly to the sheep industry. It had spread into these areas through two main pathways (Figure 1.1). The first main pathway was the eastern to western movement of BTV -1, -4, -9 and -16 originally affecting the Greek islands and moving into the Balkan regions as well as the Mediterranean islands of Corsica, Sicily and Sardinia. The second being the southern to northern expansion involving BTV -2,-3 and recently BTV-1 from Algeria, Tunisia into mainland Italy, the Spanish Balearic islands and finally mainland Spain and Portugal (Purse *et al.*, 2005).

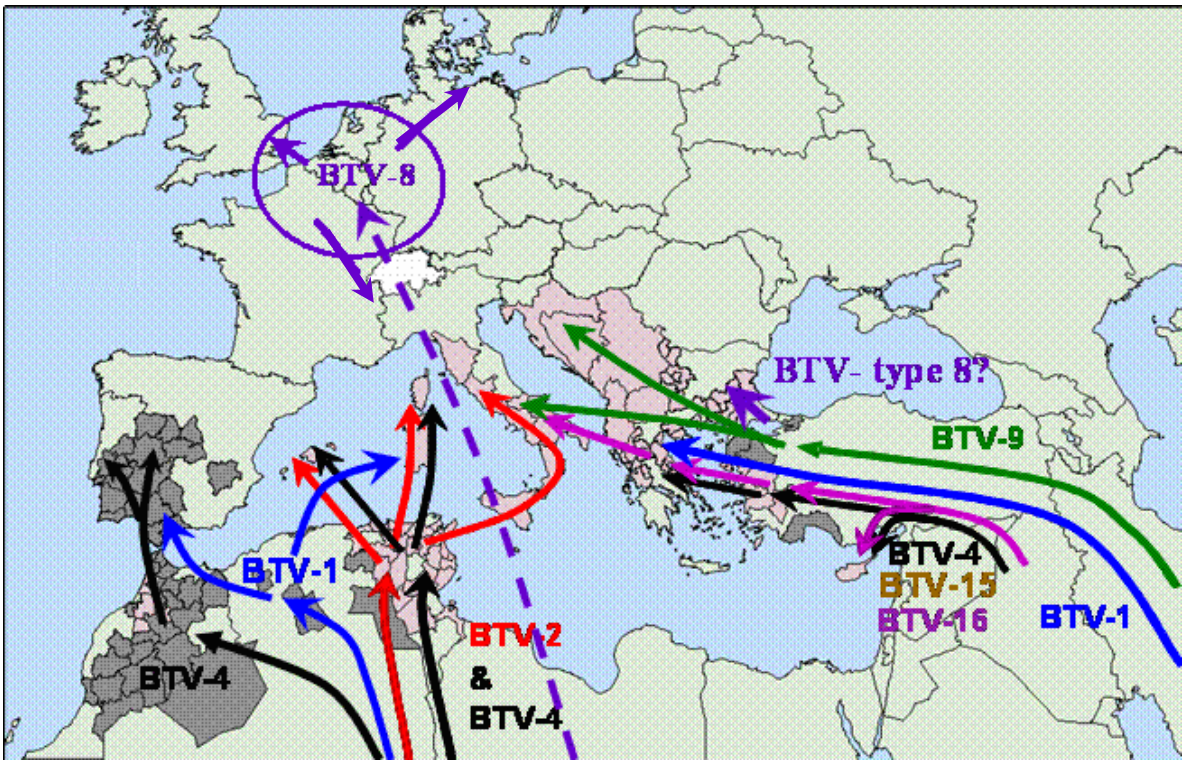

Figure 1.1: The spread and establishment of various BT serotype in the last decade in Europe (modified after figure published by the Institute of Animal Health, Pirbright UK, www.iah.bbsrc.ac.uk/bluetongue/)

Bluetongue virus serotype 8 appeared in northern Europe in 2006, firstly being reported in the Netherlands and Belgium, followed by Germany, Luxembourg, and northern parts of France (Mehlhorn *et al.*, 2007). During the winter of 2006-2007, no further cases were detected, yet in spring of 2007 BTV-8 was discovered again in sentinel animals in Germany. It was consequently reported in the same countries as the precedent year, with the additional spread into south-eastern England, Denmark and was detected in the northern part of Switzerland at the end of October 2007 (Hofmann *et al*., 2008).

Bluetongue disease is exclusively related to the presence of the virus in the competent vector: insects of the *Culicoides* species. Depending on the geographic area, BT is spread by different species of *Culicoides* midges (see table 1.2). These vectors belong to the order diptera in the ceratopogonidae family, where currently there are circa 1,340 species known (Mellor *et al.*, 2000).

The threat of BT re-occurring in 2007 was thought decreased due to the effect of the winter season on *Culicoides* activity and survival, since below certain temperatures (circa 12°C) there is virtually no virus replication possible in the midge (Paweska *et al.*, 2002). Yet as shown early on in this year, BT cases reappeared in previously affected areas. There are no concrete theories on the mechanisms of BT over-wintering yet a few hypothesis have been mentioned: i) the virus in the insect remains dormant in the colder periods, along with prolonged survival of the midge in colder temperatures (Losson *et al.*, 2007), ii) the immune system of the host allowing for the virus to remain active until the next vector season (Takamatsu *et al.*, 2003) or iii) the possibility of transovarial transmission as seen in many arboviruses and their invertebrate vectors (White *et al.*, 2005).

Bluetongue disease in Switzerland

At the time of writing, BTV-8 was reported in northern Switzerland in the canton of Basel-Stadt. A few days later a further farm was affected in the canton of Solothurn, followed by a case in Basel-Land (www.bluetongue.ch). To date, a total of 12 cattle and two goats have tested positive for BTV-8 on seven different farms. Although no firm conclusion have yet arisen as to the cause of these cases, wind direction patterns along with temperature records of these areas suggest possible intrusion of infected vectors originating from the surrounding BT affected areas.

Table 1.2: Some *Culicoides* species/groups (BT vectors) and their known geographic distribution. (Source modified from www.fao.org, and Institute of Animal Health, Pirbright)

***Culicoides* species**	**Distribution**
Culicoides dewulfi	Europe, Russia, Scandinavia
Culicoides imicola	Africa, Middle East, Mediterranean area, South Asia and East Asia
Culicoides obsoletus (meigen)	Area covering Europe, Scandinavia, Russia, Japan, Northern Africa through to Israel
Culicoides pulicaris	Palaearctic region, Pakistan and Middle East
Culicoides homotomus	China
Culicoides oxystoma	China
Culicoides actoni	Australia, South East Asia, East Asia
Culicoides brevitarsis	Australia, South East Asia
Culicoides brevipalpis	Asia, Australia
Culicoides wadai	Australia, South East Asia
Culicoides variipennis	Canada, North America
Culicoides insignis	South, North and Central America
Culicoides pusillus	South, North and Central America
Culicoides filariferus	Central America, Caribbean
Culicoides bolitinos	South Africa
Culicoides cornutus	South Africa

Bovine Anaplasmosis

Anaplasmosis in cattle is caused by a ricksettia-like organism, *Anaplasma marginale* belonging to the family *Anaplasmataceae* which parasites the hosts' red blood cells (Kuttler, 1984). It is the most prevalent tick-borne pathogen of cattle worldwide and is endemic in tropical and subtropical regions of the world. Cattle of any age are susceptible yet the severity of the disease generally increases with age and can reach morality rates of 30-50% in animals showing clinical disease. *Anaplasma marginale* can also infect whitetail deer, goats and sheep as well as other ruminant on a subclinical level. Cattle can also develop anaplasmosis through another agent

known as *Anaplasma centrale*, yet clinical outbreaks caused by the latter are generally rare. Clinical symptoms in cattle range from anaemia, depression, fever, anorexia and difficulty in breathing (www.fao.org). Additionally, excitability and aggression are frequently reported due this breathing impairment. The incubation period after an infective tick bite is believed to be about 1-3 months, and unless treated, recovered animals remain carriers for life and serve as a source of infection to other hosts which are less resistance to clinical disease. Morbidity data ranges from country and bovine species, but has shown that it can occur on a sporadic manner with a few severe cases in a herd, or it can affect a large number of animals which depends on the general level of herd immunity and prior exposure to the parasite.

Anaplasmosis is also transmitted in a variety of mechanical ways for example through vaccination, tattooing or castration tools, yet the main method of spreading disease is through several species of ticks which act as biological vectors. Geography and climate are the two important factors determining what tick species are responsible for local cases of Anaplasmosis. In Europe, the tick species *Ixodes ricinus* is thought to be the most important vector for the transmission of both *Anaplasma marginale* and *Anaplamsa phagocytophilum*, both causing emerging infections in temperate parts of the globe as seen in table 1.3. In the United States, the following ticks are believed to transmit the disease: *Dermacenter andersoni, Dermacenter variabilis, Boophilus annalatus, Argas persicus,* and *Boophilus microplus,* with the latter species also being responsible for disease in northern and eastern parts of Australia.

Table 1.3: List of tick species causing bovine anaplasmosis and their geographic distribution.

Tick vector species	***Anaplasma* species**	**Distribution**
Ixodes ricinus	*A. marginale*	Europe, North Africa
Boophilus microplus	*A. marginale*	Tropics and subtropics
Rhipicephalus simus	*A. centrale*	Africa

Although identification of the organism is important especially due to similarities between anaplasmosis and babesiosis, a disease often present in the same region, finding agents in blood samples after the initial acute parasitaemic episode is difficult. Thus confirmation of disease through serological tests, mainly enzyme-linked immunosorbent assays (Competitive ELISA) or card agglutination tests (CAT) is often needed. Once disease has been confirmed, animals can be treated with tetracycline (www.oie.int).

Anaplasma marginale in Switzerland

In 2002, a severe outbreak of anaplasmosis was reported in the eastern part of Switzerland in the canton of Grisons in a large cattle trading farm. This culminated in the culling of 300 cattle (Brulisauer *et al.*, 2004). Of all the animals tested in this outbreak, 47% were positive for *Anaplasma marginale* (Hofmann-Lehmann *et al.*, 2004). Since then, several studies have been conducted and have ruled out any emerging disease yet confirmed the presence of *Anaplasmosis* agents in Switzerland.

Bovine Babesiosis

Bovine babesiosis is caused by several protozoan agents mainly *Babesia (B.) bovis* and *B. bigeminaas* but also by *B. divergens*, *B. major*, *B. ovate*, and *B. jakimovi.* Bovine babesiosis is a tick–borne infection with high mortality and morbidity rates, and amounts to significant economic losses. Similarly to anaplasmosis, it is commonly found in tropical regions of the globe but is also heavily present in parts of Africa, Australia, Southern and Central America as well as in southern Europe. *B. divergens* however is the main cause of bovine babesiosis in northern and central Europe. Clinical symptoms are similar to those of bovine anaplasmosis, and range from anorexia, muscle tremors, haemoglobinuria, depression and fever (Preston, 2001). Abortions might also occur in heavily affected cases. As with anaplasmosis, cattle with babesiosis might show respiratory distress and eventually also signs of irritability and aggression.

As with most vector-borne diseases, the geographic distribution of the disease depends on the presence of the vector. In Australia and in tropical and subtropical regions, the main vector responsible for the disease is the tick species *Boophilus microplus*. In Europe, *Ixodes ricinus* is responsible for transmitting the disease (Moreno and Estrada-Pena, 1997) as seen in table 1.4. Once bitten by an infected tick, symptoms appear after two to three weeks, yet this also depends on the agent involved and the magnitude of the tick infestation.

Table 1.4: List of tick species causing bovine babesiosis and their geographic distribution.

Tick vector species	***Babesia* species**	**Distribution**
Ixodes ricinus	*B. divergens*	Europe
Boophilus microplus	*B. bigemina* and *B. bovis*	Tropics and subtropics

Apart from the identifying the agent through microscopic blood smears, diagnostic procedures also include serological testing when the concentration of parasites in the blood is not large enough to detect. Yet further testing is needed when detecting certain agents due to the high occurrence of cross reactions, especially in the case between *B. bovis* and *B. bigemina* when using the indirect fluorescent antibody test (IFAT) (www.oie.int).

Babesia divergens in Switzerland

Several studies have been conducted in Switzerland on ticks and the disease they transmit. In the analysis of 700 ticks in the study by (Hilpertshauser *et al.*, 2006), six *Ixodes ricinus* were found to have *Babesia divergens*, albeit at low prevalence levels.

The last large outbreak of bovine babesiosis occurred in combination with other tick borne diseases, in the same cattle trading farm in eastern Switzerland as mentioned above (Brulisauer *et al.*, 2004). This outbreak was surprising due to the number of co-infections present in the cattle. From the animals tested, 90% had up to five other infectious agents present including *Theileria* spp, *Anaplasma marginale*, *Anaplasma phagocytophylum* and *Mycoplasma wenyonii*, of which certain had never been reported in Switzerland (Hofmann-Lehmann *et al.*, 2004).

1.3 Vector biology

Midge biology

Similar to *I. ricinus*, species belonging to the BT vector of *Culicoides* are also able to transmit a variety of diseases such as: African horse sickness, Epizootic haemorrhagic disease, Akabane disease, Equine encephalosis and Ephemeral fever. And as seen in Table 1.2, they have an extensive distribution. Again this is limited to climatic and geographic factors with precipitation, relative humidity, altitude and most importantly temperature playing a role in their survival and activity rates.

The exact life cycle (Figure 1.2) of different *Culicoides* varies, but in general includes four larval instar stages which can last from a few days to several months (including diapause if

process happens during winter months). Pupation then occurs for a period ranging from two days to a month, followed by the adult life stage. The complete life cycle can occur in two to six weeks, all dependent as mentioned, on the species involved as well as the environmental conditions. The amount of eggs produced by the female again varies among species, and can range from 25-300 eggs.

Transmission of BT occurs when the female adult bites a host since blood meals are necessary for the development of eggs. If either the host or the vector is infected, there is a possibility of BT transmission, albeit at different rates depending on host and vector species, host immune status, as well as climate dependent factors.

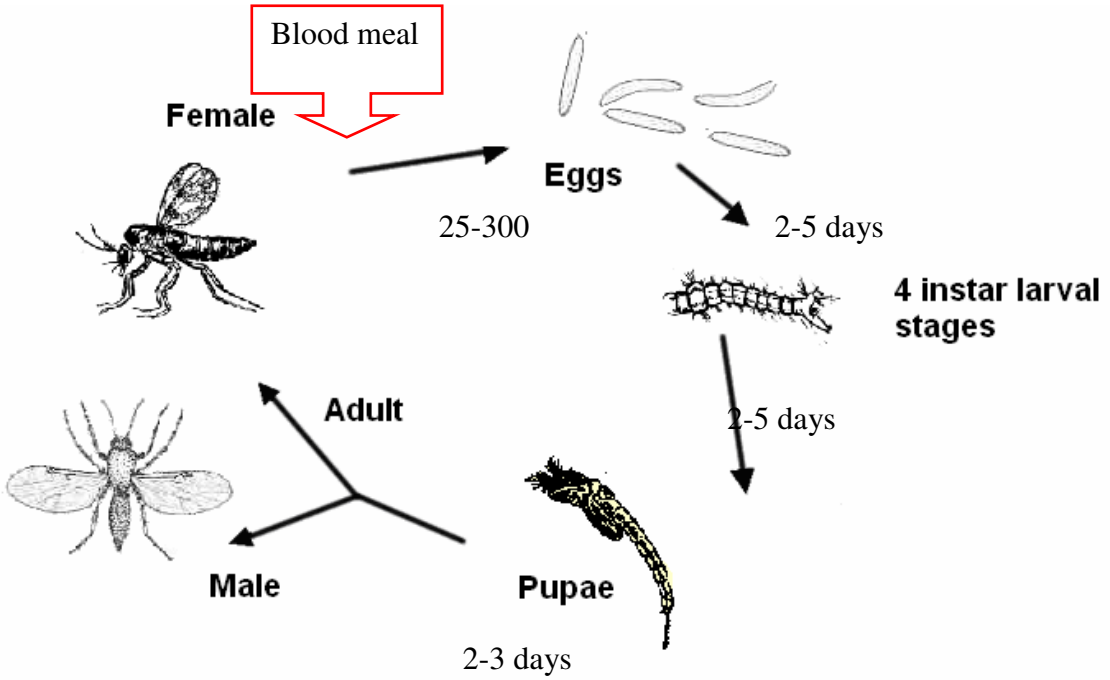

Figure 1.2: General life cycle of *Culicoides species*. Source modified from (Purse *et al.*, 2005)

Culicoides vectors in Switzerland

As previously mentioned, climate changes, especially their effect on the *Culicoides* vectors are significant in the spread of BT. The potential for the establishment of *Culicoides* species competent to transmit BT in higher altitudes than previously recorded is especially concerning for Switzerland. The effect of global warming is said to be most apparent in higher latitudes in the Northern hemisphere whereby temperature levels could be rising by 10°C (Watson *et al.*, 2005). In contrast, it is arguable that the more extreme low temperatures being

seen on a global scale in the winter seasons could limit the vector reproduction and survival factor, yet Europe had two successive mild winters in 2005 and 2006, which helped propagate the current expansion of BT in certain areas.

Culicoides midges had been studied in northern Switzerland in the canton of Basel (Habermacher, 1984), in the southern canton of Ticino (Racloz *et al.*, 2006b), as well as a general survey on the population as seen in (Cagienard *et al.*, 2006a) in 2003. The number of *Culicoides* species ranged from 17 as described in the *Diptera* checklist of the *Fauna Helvetica, (*Merz et al., 2002), to 22 (Habermacher, 1984), on separate accounts, yet summarizing the findings of other studies, it is reported that 35 *Culicoides* species were found in the country. Importantly, this list is compromised of five species currently thought to transmit BT disease: *C. obsoletus* (sensu stricto), *C. scoticus*, *C. chiopterus*, *C. pulicaris* and *C. dewulfi*. There has also been the identification of a single *C.imicola* in southern Switzerland in 2003 (Cagienard *et al.*, 2006a) , yet to date is considered of negligible epidemiological significance.

Tick biology

The tick species *Ixodes ricinus* is not only key for the transmission of the causative agents of Anaplasmosis and Babesiois and other species such as *Ehrlichia phagocytophila* and *Coxiella burnetii*, but is able to spread Lyme disease, louping ill, Crimean Congo hemorrhagic fever, tick–borne encephalitis, Bukhovinian hemorrhagic fever and rickettsial tick borne fever of sheep (www.oie.int).

Although distributed worldwide, *I. ricinus* is limited to areas which have considerably high levels of relative humidity (above 70%) due to their vulnerability to desiccation, as well as being constricted to areas bordering woodland and shrubs. Altitude also plays a role in its distribution whereby their occurrence is rare above 1600 meters above sea level (Estrada-Peña, 2006).

Belonging to the *Ixodidae* family of hard ticks, *I. ricinus* is a three host stage tick which takes two to four years to develop into a mature adult, whilst generally taking one year for each life stage. This implies that at each life stage, the tick will target a different host since it needs a blood meal at every stage, which lasts for several days (Figure 1.3). Primarily, eggs are laid on the ground, and when hatched, the larvae feed on small vertebrates (i.e. rodents or rabbits). Once they develop into nymphs, they parasitize middle sized mammals, for example foxes, dogs or

cats. Finally, mature adults prefer to feed on larger mammals such as humans, deer, cattle and sheep.

Transmission of *Babesia divergens* can occur when the tick parasitizes its host for a blood meal, and can also persist in the vector through vertical transmission.

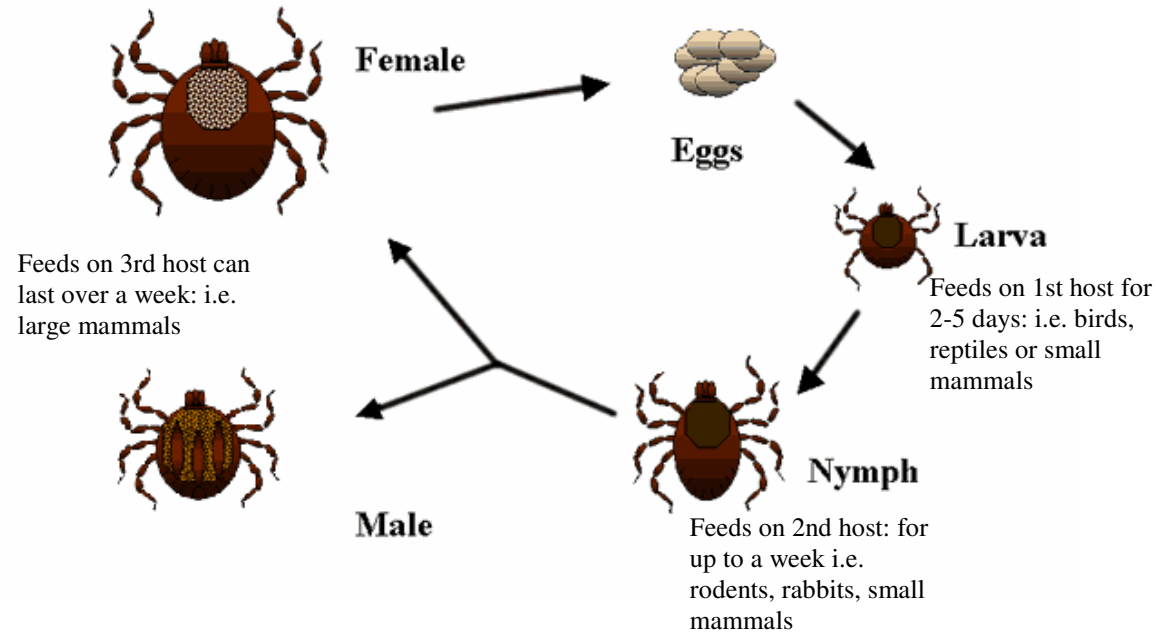

Figure 1.3: General life cycle of *Ixodes ricinus*. Source modified from www.pathmicro.med.sc.edu /parasitology /ticks.htm

Ixodes ricinus in Switzerland

As mentioned, many studies conducted in Switzerland have resulted in confirming the established presence of *Ixodes ricinus.* Another study conducted in southern Switzerland reported that out of 2,017 ticks caught, 99.2% were *Ixodes ricinus* (Hilpertshauser *et al.*, 2006). A further study by Casati *et al.*, 2006, conducted in larger parts of Switzerland also found this tick species distributed throughout the country. This tick species has been identified in the cantons of Zurich, Ticino, Neuchatel, Bern, Valais, Grisons as well as being found on migratory birds (Marie-Angele *et al.*, 2006). Through these results, it is assumed that this tick species is indigenous in Switzerland.

1.4 Surveillance of vector-borne diseases

Summary of surveillance systems

Veterinary surveillance is described as surveying the occurrence of a disease and its status in the animal population (Racloz et al., 2006b, Salman et al., 2003). It includes the collection, analysis, and interpretation of a variety of data for purposes of improving animal health and safety. A part of a surveillance program can also contain the monitoring of a disease and includes identifying changes in disease prevalence levels or identifying its spread. Depending on the nature of the disease involved along with its status in a country or region, different types of surveillance systems exist. A surveillance system can be based on two basic approaches: passive and active surveillance. Passive surveillance is usually described as the reliance on external sources to gather information, whilst active surveillance involves a more dynamic approach in collecting the necessary data.

One of the methods for active surveillance is called "sentinel herd surveillance". A sentinel herd is described as 'a cohort of animals at a pre-determined location, which is monitored over a specified period of time with respect to a specified disease agent' (Ward *et al.*, 1995). Sentinel herds are usually carefully selected to be located in high-risk areas of disease occurrence or likely vector establishment. Such an approach is categorized as a targeted surveillance system as opposed to random surveillance (Stark *et al.*, 2006).

Sentinel herd surveillance in Switzerland

Apart from the Swiss Sentinel Surveillance Network (SSSN) created in 1986 for the monitoring of a range of public health related diseases and affections, (such as asthma, measles and rubella) (Paget *et al.*, 2000), and the use of sentinel water-birds for the surveillance of Avian Influenza in northern Switzerland (www.projekt-constanze.info), the use of this active system have not been widely publicised.

One of the advantages of a sentinel herd surveillance is its ability to act as an early warning system. This was considered important especially for diseases which were considered rare or emerging. Hence a bluetongue sentinel herd system was established in 2003 involving

serological blood sampling of cattle as well as an entomological sampling component (Racloz *et al.*, 2006b).

1.5 Background of methods used in thesis

Use of Geographic Information Systems in disease surveillance

Applications of Geographic Information Systems (GIS) have been used in a variety of ways especially in the past few years due to the development and improvement of specialised software programs such as ArcGIS (Version 8.3, Environmental Systems Research Institute, Inc.). The facility to analyse data in both a spatial and temporal way and to visualise it through mapping techniques has contributed to epidemiology by expanding knowledge on disease information and patterns both retrospectively and prospectively. For example, remotely-sensed data derived from satellite images, as well as land-surface or climatic data can be used to predict the potential spread of a disease, or analyse what parameters dictate the presence of a vector. Various studies have used GIS as either a visual aid or as a modelling tool (Pfeiffer and Hugh-Jones, 2002) with examples ranging from bluetongue modelling in Corsica based on landscape ecology (Guis, 2007) or maps integrating vector dynamics, host distribution and the transmission of disease in a study on Rift Valley Fever in Africa (de La Roque et al., 2007).

Basic reproduction number (R_0) calculations for vector-borne diseases

As discussed in chapter 8, the basic reproduction number (R_0) for vector-borne diseases is a more complex number to calculate due to the influence of seasonal fluctuations (Bacaër, 2007), local climate and environmental features as well as the abundance of breeding sites available near hosts which affect vector dynamics (Lord *et al.*, 1996). The basic reproduction number is described as the 'expected number of secondary cases that would arise from a typical primary case in a susceptible population' (Roberts and Heesterbeek, 2003). The transmission rates of the disease will also change depending on temperature factors affecting vector to host or host to vector interaction, along with the extrinsic incubation time, biting rates and vector mortality rates (Gubbins *et al.*, 2007). In this study, vector density numbers along with temperature values were used from field data collected throughout the project in order to produce R_0 values specific to the areas and time frame studied.

CHAPTER 2

Study aim

The aim of this study was to develop and implement a sentinel herd surveillance program with the main purpose of serving as an early warning system for selected vector-borne diseases in farm animals. This would be through:

1) Determining the immune status of the selected sentinel herds in terms of the following three diseases: BT, cattle anaplasmosis and cattle babesiosis. (Discussed in chapter 5)

2) Study the abundance, distribution and population composition of BT vectors in Switzerland. (Discussed in chapter 7)

3) Identifying areas of higher risk for disease incursion as well as vector establishment. (Discussed in chapter 6 & 8)

CHAPTER 3

Review of sentinel surveillance systems with special focus on vector-borne diseases

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This paper has been published in Animal Health Research Review
2006 Jun-Dec; 7 (1-2):71-9

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Abstract

In the past few decades, vector-borne diseases have been spreading into countries previously free of these agents. It is necessary for a surveillance method to be tailored to the biology of these agents in order to detect their incursion. Using a sentinel herd system, it is possible to target high-risk areas where occurrence is most probable due to vector presence. Since the 1970s, diseases such as Akabane, Vesicular Stomatitis and Bluetongue disease have successfully been monitored using cattle herds as sentinels in many countries such as Saudi Arabia, Australia, China, Indonesia, Sultanate of Oman and most recently in countries in Western Europe.

This paper reviews the strengths and weaknesses of sentinel herd surveillance systems in general. In order to determine their efficacy, the following criteria were found to be essential: the choice of sentinel locations, sentinel animal, seasonality of sampling, and diagnostic testing methods. We conclude that due to its ability to focus on a certain disease, sentinel herd systems have been successful in the early detection of the spread of a targeted agent. This review is used as a basis for recommendations for the development of future sentinel herd systems.

Keywords: Vector-borne disease, sentinel herds, early warning, surveillance

Introduction

In order to maintain healthy wildlife and livestock populations, it is important to control and prevent diseases from occurring and spreading. Due to the variety of veterinary diseases circulating worldwide, it is important to have a disease monitoring and surveillance system tailored to the pathogen in question. Over the past few decades, vector-borne diseases have been emerging into geographic areas previously free of such infections. Malaria, Rift Valley fever, Dengue fever, Ross River virus, Murray Valley encephalitis, Schistosomiasis and Japanese encephalitis are examples of diseases where factors such as climate change, intensification of agriculture and urbanization have led to an increased incidence, especially in developing countries (Sutherst, 2004). Other factors aiding this emergence is the expanding pattern of tourist travel, as well as animal movement (Anonymous, 2005). This propagation is sustained by the fact that in the last decade, environmental changes through global warming have created new regions for vector-borne diseases to establish themselves (Gubler, 1998). Since these diseases were historically contained in other regions of the world, there is little evidence or experience on how the disease or its vector will behave in a new surrounding with different habitats, climates and susceptible hosts.

By studying livestock disease patterns and their dynamics, various surveillance strategies have been devised and applied. They share the aims of minimizing the effects of a disease upon a population, as well as preventing its spread to surrounding areas either within national borders or internationally. Animal disease surveillance has two main purposes, one of which is its use as a tool in assessing the health status of a population. This includes the detection of emerging exotic diseases and their vectors, as well as monitoring the shift in endemic disease prevalence. The second purpose is to determine the effectiveness of a specific control strategy that has been created to manage the disease, for example by routine sampling at slaughter houses in a national disease eradication program.

Surveillance is described as a systematic collection, analysis and interpretation of disease-related events occurring in a population, allowing for the implementation and planning of control measures subject to the results obtained from the surveillance system (Anonymous, 2004). Several types of surveillance methods exist, and are classified according to their function and data collection method (Thrusfield, 2005). *Passive* surveillance is defined as a fixed, routine method which typically involves examining clinical cases, and usually relies on veterinarians and

farmers to report suspicious cases. The disadvantages of passive surveillance are factors such as under-reporting and selection bias depending on the data source. *Active* surveillance can include the sampling of clinically normal animals and entails a more active and purposeful cooperation from stakeholders involved. Surveys, sentinel systems and mass screening methods are examples of active surveillance. Surveillance can also be categorized due to the way the observation units are chosen: probability (random) or non-probability (non-random) sampling. Random sampling involves the selection of the sampling unit in an unbiased a manner as possible, so that each unit has an equal chance of being chosen. Non-random sampling includes strategies such as risk-based, targeted and sentinel surveillance. In these surveillance types, the sampling units are selected primarily by the investigator and are based on choosing a specific unit to suit the objective of the study.

The term 'sentinel' originates from the Latin word, *sentire*: to feel and transformed itself into *sentina*: Italian for vigilance and finally, into *sentinelle* in French. In epidemiological terms, a sentinel herd is defined as a cohort of animals at a pre-determined location, which is monitored over a specified period of time with respect to a specified disease agent (Ward *et al.*, 1995). As explained by McCluskey, sentinel surveillance is used '*to monitor or identify outbreaks and epidemics caused by infectious agents, to investigate changes in prevalence or incidence of endemic or infectious agents, to evaluate the effectiveness of newly instituted disease control programs, and to confirm a hypothesis about the ecology of epidemiology of an infectious agent'* (Salman, 2003). According to the World Animal Health Organization (OIE), sentinel units are described as the regular testing and identification of animals whose geographical location and immune status is known, in order to detect disease occurrence. The data collected can provide information on the local incidence rate or prevalence as well as prove the freedom of infection status of the specific pathogen under investigation.

The establishment of a sentinel herd system allows for a targeted surveillance using risk-factor knowledge. The term targeted surveillance involves testing certain animals of a sub-population where disease is more likely to be introduced or found, and is part of a risk-based surveillance strategy. A proposed definition for risk-based surveillance has been described as *'a surveillance programme in the design of which exposure and risk assessment methods have been applied together with traditional design approaches in order to assure appropriate and cost-*

effective data collection' (Stark *et al.*, 2006). It can be applied to a wide range of diseases and conditions such as animal welfare, endemic or exotic, infectious as well as vector-borne diseases.

This review aims to describe the various criteria used in selecting sentinel animals, location and sampling strategies involved for a variety of diseases, as well as determining the factors for assessing the efficacy of sentinel herd systems as a surveillance method. This has been accomplished through the collection of peer-reviewed articles and studying documentation of national surveillance programs. Recommendations for use of sentinel herd surveillance and its success factors, as well as its disadvantages will also be discussed.

Application

Sentinel herd surveillance has been regularly used over the past few decades covering a wide variety of diseases and conditions. Studies using sentinel herds have been conducted for parasitic, viral, bacterial and vector-borne diseases as well as for toxicological screening and animal welfare issues. Examples of established sentinel herd surveillance programs are shown in Table 3.1. Although sentinel herd surveillance has been used for such a broad range of diseases, the basis of creating a sentinel herd as a surveillance tool remains similar in all cases. It consists of two parts: firstly establishing the objective of the surveillance, and secondly, deciding upon the specific selection and design criteria needed depending on the nature of the disease/condition in question.

Establishing the objective of the sentinel herd surveillance

Whether a disease is endemic to a region or country, or is considered exotic, sentinel herd systems can be tailored specifically to the type of surveillance needed. Sentinel herd surveillance can be divided into two main branches (Fig. 3.1). Firstly, to measure the frequency of an existing disease within different study objectives. Three main objectives within this group were; 1) to monitor the occurrence or to determine the dynamics of a specific disease, 2) testing a control strategy for a specific disease and, 3) assessing exposure risk. The second branch of sentinel herd surveillance is its use as an early warning tool for either; 1) detecting first incursion of a disease or its vector into previously free regions, or 2) detecting its return to that area (Fig. 3.1).

Once the objective of the sentinel herd has been established, design and selection criteria need to be defined in order to fit the disease, condition, vector or agent under investigation. This includes defining the selection of a specific region, sentinel animal species, characteristic of the herd, and finally determining the type and frequency of testing (Salman, 2003) (Fig. 3.2).

The choice of location largely depends on the actual purpose of the sentinel surveillance. If the main objective is the surveillance of an existing disease, this would imply choosing a location where the disease is known to circulate, and therefore non-random. If the disease is endemic, the choice of sentinel herd can be random. Furthermore, the sentinel animal within the herd can then be randomly chosen, granted it is susceptible to infection. If the goal of the surveillance is to serve as an early warning system, it is important to choose a location considered as a high-risk zone for incursion of the agent or vector. The identification of high-risk zones is essential since missing the first incursion of disease or its vector could have serious consequences for the rest of the country, as well as rendering the system ineffective. Vector-borne diseases occur in areas where the vector can establish itself, therefore high-risk zones can be identified depending on climatic and geographic factors as well as actual vector presence (e.g. determined by entomological trapping methods). The selection of the sentinel location is therefore non-random, although the choice of sentinel animal within this herd can eventually be random. Different regions represent varying levels of disease/vector exposure risk to the susceptible host population. Therefore, depending on the study objective, the decision on choice of herd location needs to take this into consideration. Exposure risks depend on a variety of factors including production purpose, or type of husbandry management in place (e.g. livestock kept on the same farm have different levels of exposure risk when compared to cattle displaced to alpine pastures during the summer months). In terms of vector-borne diseases, the exposure-risk would be the time spent in areas where vectors are present.

Selecting the sentinel animal species requires epidemiological knowledge of the disease in question. As a minimal rule, the sentinel animal species must be susceptible to the disease and be able to generate a measurable response, preferably more easily detectable than in other species susceptible to the same disease. Selection criteria concerning the age of the sentinel animal vary depending on the aim of the surveillance. If the incidence of the disease is desired, one could designate younger animals as sentinels, since they have only been on the farm for fewer (vector) seasons, hence exposure is limited.

Depending on the seasonality, mode of transmission and severity of the disease, the testing period and diagnostic methods used will differ in each case. Surveillance of an exotic or highly pathogenic infection will need more frequent sampling, whilst testing for a vector borne disease generally takes place before and/or after the vector season has occurred. The timing will depend on the epidemiology of the disease.

The particular difficulty for the establishment of an appropriate surveillance system concerning vector-borne diseases is that in addition to determining the objective of the surveillance and selecting the set-up criteria, the actual biology and consequent ecology of the vector have to be taken into consideration. This implies acquiring background knowledge on the geographical and climatic preferences of the vector and potential risk factors involved in terms of their occurrence and/or establishment into an area where susceptible hosts are located. Seasonal dynamics, landscape features, meteorological data and host preference are certain factors to be aware of when selecting the location of the sentinel herd for a vector-borne disease. An additional challenge is that a certain agent might be transmitted by different vectors depending on the area in which it is located in, thereby resulting in variation in sentinel herd strategies for each specific country. For example, Bluetongue disease is transmitted by different species of the *Culicoides* midge depending on the country that is involved, hence various environmental conditions need to be addressed when planning to set up a sentinel herd for surveillance of this disease(Kline and Wood, 1988);(Schmidtmann *et al.*, 2000). It is therefore advisable for a surveillance system involving a vector-borne disease to be made of two components: a sentinel herd host surveillance, and an entomological surveillance activity. This will provide for determination of the vector species composition as well as monitoring the changes in frequency and abundance in order to establish certain patterns in their life cycles for that specific region (preferably near the sentinel herd location). In the United States, this type of setting (i.e. vertebrate host and vector surveillance) has been established for diseases such as West Nile, Eastern and Western equine encephalitis, La Crosse and St. Louis encephalitis and Bluetongue disease (Gubler, 1998). The National Arbovirus Monitoring Program (NAMP) in Australia also uses this method for the surveillance of the following diseases and their vectors: Akabane, Bluetongue and Bovine Ephemeral Fever (Anonymous, 2002).

Application of sentinel herds in the context of surveillance of an existing disease

1) *Monitoring the occurrence or dynamics of a disease.*

Vesicular Stomatitis (VS) is an economically important arboviral disease of livestock, especially in horse and cattle, although the exact reservoir host has not yet been identified. In an epidemiological study conducted in Colorado, the persistence of VS was determined in a 3- year-long project (McCluskey *et al.*, 2002). Twenty sentinel locations in the state of Colorado, USA, were visited and clinical examinations as well as serological tests were carried out. The selection criteria for the location of the sentinel herd were based on the previous presence of VS on site, as well as the voluntary participation of the owner. Horses, chosen non -randomly by the owner, were used as sentinel animal species since records show that the United States has been experiencing a greater number of clinical cases in horses as compared with cattle (McCluskey *et al.*, 1999). A competitive Enzyme Linked Immunosorbent Assay (ELISA) was chosen due to its financial advantages and its high level of sensitivity. The testing strategy was conceived to identify sero-conversions, and since VS antibodies only remain in circulation for about 45-60 days (McCluskey and Mumford, 2000), sentinel animals were tested 3-4 times per year.

Another study with the aim of determining the incidence of a disease was conducted in Saudi Arabia for Akabane disease(Abu Elzein *et al.*, 1998b). Sentinel locations were chosen again depending on the recorded presence of the disease and its vector. Sentinel species included cattle, sheep and goat, all known to be susceptible carriers of Akabane disease. Serological testing was conducted at specific time points including blood sampling from dams just after parturition, and from their offspring at birth before suckling. These specific time points would provide information on the role of maternal antibodies in Akabane disease transmission. Therefore the selection criteria of location, species and test strategy for this sentinel herd system were also tailored to suit the study objective and the epidemiology of the disease in question.

2) Testing the efficacy of a control strategy for a specific disease.

Substantial trade restrictions resulted from the presence of certain parasites in the deer industry in New Zealand. In this example, sentinel herd surveillance was used in order to evaluate

different internal parasite control strategies (Audige *et al.*, 1998). As in the case of studying the dynamics of a disease, the actual presence of the disease is required when testing a control strategy. Sentinel farm locations were chosen by convenience, but were able to represent the typical deer farming conditions in that area. Individual sentinel deer were randomly chosen by the research group to avoid farmer bias, and grouped by sex and age to receive anthelmintic treatments. The testing strategy involved blood sampling as well as faecal egg and larval counts collected in spring, summer, autumn and winter. This allowed for the collection of samples throughout four different seasons as well as corresponding to deer reproduction patterns. The laboratory test used was the standard screening method for veterinary purpose available at that time. A different approach involving the use of sentinel herds to test a control strategy was applied in a study to determine the effect of acaricide control on the island of Nevis in the Caribbean. A sentinel herd of cattle (*Bos taurus*) was used as a control group to determine the efficacy of acaricide treatment (in a group of similar cattle) for controlling bovine dermatophilosis (Hadrill and Walker, 1994). The choice of the sentinel species was supported by evidence that *B. taurus* are considered more susceptible to tick bites than other cattle breeds in similar geographic regions(Tatchell and Moorhouse, 1968). The testing procedure involved weekly counts of adult ticks and ranking clinical symptoms on the sentinels compared to the other cattle undergoing acaricide treatment.

A similar approach was used to test treatments against African Animal Trypanosomiasis (AAT) in a study conducted in Burkina Faso (Bauer *et al.*, 1992). The sentinel animals underwent initial treatment before the study was conducted and were used as a control group to measure the efficacy of flumethrin pour-on in cattle.

In contrast, a different approach to test the efficacy of a control strategy is the incorporation of sentinel animals into a vaccinated herd. An important factor to combat Avian Influenza is the ability to differentiate vaccinated poultry from infected poultry and birds. It was envisaged to apply this strategy recently in certain regions of France (the departments of Landes, Loire-Atlantique and Vendée), as part of a monitoring program. The aim of the vaccination was to reach 90,000 birds by the beginning of April 2006 and included a pre-vaccination examination of flocks to ensure health and bio-security standards, monthly clinical surveillance of the holdings, and finally the use of non-vaccinated sentinel birds to detect any outbreaks in the

vaccinated flock. A similar method was planned for a Dutch vaccination campaign applied to hobby poultry and to free-range laying hens throughout the whole country (Anonymous, 2006).

3) Sentinel herd used to estimate exposure risk.

Similarly to the two previous objectives, the selection of the sentinel region to assess exposure risk needs to be based on previous recorded disease presence. Lyme disease is found in many areas of Europe and the United States and represents one of the most reported tick-borne diseases in the latter country. Due to their behaviour and close interaction with humans, dogs have been reported to be effective sentinel animals to determine human risk of Lyme disease in certain states of America (Duncan *et al.*, 2005). Based on convenience sampling, canine serum samples were tested using a very specific and sensitive ELISA test. The aim of the study was to determine whether dogs could be used as a risk indicator of disease occurrence in the human population in similar geographic locations. Sentinel dogs originated from 'hot-spot' states where previous history has shown a high occurrence of the disease, mostly in the eastern coast of the United States.

Another example of the use of a sentinel herd as an exposure assessment tool was conducted in a study to determine the risk associated with the possible transmission of diseases through xenotransplantation (Iverson and Talbot, 1998). Sentinel pigs were used to monitor herd health in order to ensure the absence of pathogens in any cells, tissues, or organs, that might potentially be transplanted into an immunosuppressed human recipient. Individual pigs were randomly chosen to be representative of their population including a variety of ages and both sexes. Direct contact between the sentinel and non-sentinel pigs in the herd, (as well as with excreta) was important in order to maximize the potential exposure of the sentinels to any pathogens that may have been present (Iverson, pers.comm.).

Application of sentinel herds as an early warning system

1) Surveillance in disease-free areas

In disease-free areas, it is important to locate zones where the first incursion of the disease/vector is most probable, as opposed to endemic diseases whose presence has been

extensively recorded. Bluetongue disease is a vector-borne animal disease of economic importance which has occurred in epidemic proportions since 1998 throughout the Mediterranean Basin and Western Europe (Purse *et al.*, 2005). Due to its presence in countries adjacent to Switzerland, a nationwide serological survey was conducted in 2003, which involved the serological sampling of randomly selected cattle farms (Cagienard *et al.*, 2006b), as well as the establishment of entomological trapping at predetermined 'risk-sites'. Risk-sites were chosen mainly on the basis of climatic and geographic factors that limited vector biology, such as average yearly temperature and altitude respectively. Based on these results, sentinel herds were selected for annual serological sampling with the aim of creating an early warning system to detect the primary incursion of the agent before substantial spread could occur. Cattle were chosen as the target species primarily since they act as the reservoir species for the BTV in sheep as well as presenting an earlier antibody response post-infection (Anonymous, 2004). Cattle farms are more abundant and more widely distributed in Switzerland and, finally, it has been shown that *Culicoides* midges have a feeding preference for cattle compared to sheep(Nevill, 1978). Another use of sentinel herd surveillance as an early warning system is to prevent the spread of BT disease into new areas such as the system used in Australia. Sentinel herds have been chosen to outline the 'possible activity' zones which cover all eight states (NAMP, 2004). From 2003-2004, 84 sentinel cattle sites and 103 vector trapping sites were monitored. Through the focused surveillance of northern Australia, it allowed for the detection of new incursions as well as being an early warning tool for monitoring spread into southern areas especially since BTV has not occurred in major commercial sheep flocks.

2) Surveillance to identify re-emergence of disease

When the purpose of the sentinel surveillance is to serve as an early warning system for a disease which has already affected an area previously, the mainstay function is to detect its re-emergence as rapidly as possible. Since 1999, West Nile (WN) virus has been recorded in the United States and is now considered endemic. In order to guide public health action in relation to the disease, the use of crow deaths as sentinels was recorded as an early warning system for human cases (Eidson *et al.*, 2001), (Anonymous 2005b). Crows were used as sentinel animals, firstly by coincidence (other bird species were not studied in enough detail at this point), and

secondly, since birds in general seem to be the major introductory or amplification route of this disease. Typical for studies involving wildlife, a convenience-based selection of sampling location was required. In this study, the crow death site determined the sentinel location choice. Most sightings were of individual birds as opposed to clusters which is more common in diseases such as Avian Influenza. The deaths were noted on a mapping system in order to visualize the pattern that developed. This sentinel system was useful as a possibility of determining the incursion of virus into areas as well as correlating them with human cases.

In aid of the prevention and control of Rift Valley Fever (RVF) in western Africa, sentinel herd monitoring has been set up since the year 2000 (Anonymous, 2005a). Sentinel locations were selected based on geographically representative areas. This involved determining 'hot-spots' for vector activity and included areas near bodies of water such as rivers or swamps. This is in contrast to the previous study where sentinel location was random and not dictated by specific 'risk factors'. Sheep and goat were chosen as sentinel species, with the additional criteria of an age limit and preferred sex, due to the lessened probability of being slaughtered during the study. As in several other studies, sentinel animals which sero-converted during the study were replaced by sero-negative animals in order to detect any new infections. Blood sampling was the diagnostic method used with a regular frequency of testing. In relation to climate, the animals were sampled before and during the rainy season and it was recommended that the herd size be kept at a specific number in order to facilitate testing and eventual replacements.

Merits and limitations

In economic terms, a sentinel-herd-based surveillance can be an attractive option due to its targeted characteristic as opposed to a random surveillance system. It can detect individual cases since data collection is traceable and organized. Sentinel herd surveillance can either be passive or active depending on the objective in mind, and therefore provides flexibility for the participators involved. It has been shown to be an effective method as an early warning tool, and therefore can help in the primary detection or resurgence of a targeted disease/vector. Another merit of sentinel herd surveillance is the generally voluntary participation of farmers. The cooperation is often due to the possible exchange of information, allowing for a motivated partnership and a more successful follow-up procedure. Depending on the prevalence of the

disease in a country, and the adopted sentinel surveillance strategy, it is possible to estimate and extrapolate morbidity measures/indicators for a wider population range. The main advantage of a sentinel herd system is its broad range of uses as well as its flexibility and focus.

In terms of validity, certain problems can arise, especially linked to the specific type of diagnostic test used. It is important to stress that certain immunological techniques will not be able to detect antibody presence after a certain period of time, and therefore the disease would pass unnoticed. Diagnostic tests involving antigen detection also have their limitations when considering the short circulation periods of certain agents, especially if visits to the sentinel herd are infrequent and clinical signs at the peak of the outbreak are missed, or if the sentinel species chosen is an asymptomatic carrier. Also hindering the efficiency of diagnostic procedures is when the disease under investigation is uncommon to the area or sero-prevalence is very low, whereby the specificity of the test will produce false positive results and generate a cascade of unnecessary actions. Depending on the method of sentinel animal or herd selection, data collected may present a biased finding, if it has not been carried out in a random fashion. If, on the other hand, a random selection process is decided upon, the location of the sentinel herd is not necessarily chosen for its accessibility (transport method), and emphasis is needed to ensure that samples are sent and results are received in the quickest manner possible. Although voluntary participation has its advantages, it is also more difficult to force a farmer to remain in the study, and drop-outs, or lack of follow-ups can have an impact on the reliability of the sentinel herd system. Disease can also be missed upon incursion due to unstrategic sentinel herd locations.

Discussion

It is difficult to obtain specific information from sentinel herd literature using systematic search methods (Rabinowitz *et al*., 1999). Although the term ‘sentinel herd’ retrieved circa 50 peer reviewed papers on the PUBMED search engine, actual information on selection criteria and specific details on use of sentinels was difficult to specify for certain studies. This is mostly due to the omission of details when the design and selection criteria are described. Further searches through national programs and academic institutions were required to find a more comprehensive number of sentinel herd literature sources. The possibility of comparing sentinel herd systems with other systems with similar objectives was also difficult due to the lack of matching criteria

listed in the studies. These problems were mostly overcome through personal communications with the authors who were very helpful by providing information upon request.

Based on the various objectives, the success of certain sentinel herds can be determined through the results obtained in the specific study. For a sentinel herd system belonging to the 'existing disease category', a study was conducted with the aim of calculating the background incidence level of RVF in Senegal (Chevalier *et al.*, 2005). Sentinel animals were serologically tested and the results allowed for further strategic alterations as well as proving the efficacy of this type of surveillance system.

Measuring the effectiveness of a sentinel surveillance system in terms of an early warning tool is more difficult due to the lack of comparison points. Yet recently, the incorporation of models to assist in the design and selection of sentinel herds, or to forecast disease/vector occurrence has been increasing. Climate models were created in order to predict possible Bluetongue risk zones using temporal pattern data in a study conducted in Israel (Purse *et al.*, 2004a). Another example is the study by Giovannini *et al*., where in order to plan the continuation of the sentinel herd surveillance strategy in place since 2001 in Italy, a Monte Carlo model which simulated the expected number of sero-converting animals was created to help in decision making (Giovannini *et al.*, 2004).

Despite the limitations of a sentinel herd surveillance system, it can be a very effective tool for the surveillance of a specific condition or disease category. If the aims of the study are well defined, and the selection criteria have taken in all the specific disease/vector characteristics, setting up a sentinel unit as a targeted disease monitoring tool can be very successful. To date, sentinel herd surveillance has been used for a variety of purposes such as monitoring for the presence of new or re-emerging diseases, surveying anti-microbial resistance and even as a method to prevent bio-terrorism (Brannen and Stanley, 2004). It is important to stress however, that the successful use of sentinel herd surveillance depends on the precision of targeting a disease/vector, which inevitably depends on the availability and correct interpretation of epidemiological knowledge.

In this review, sentinel surveillance has been described for different objectives ranging from determining the dynamics of a specific disease, to measuring the efficacy of their control program, as well as an early warning system for emerging vector-borne diseases. The ability to tailor the surveillance by means of the particular selection of location, sentinel species and

diagnostic method can create a valuable system for the detection of a wide range of diseases or conditions be it of welfare, endemic, exotic, infectious or vector-borne origin.

As mentioned in the merits and limitations of a sentinel surveillance system, due to the nature of sample collection and the infrastructure needed, sentinel herds are less suited for national disease prevalence surveys, yet more apt as early warning systems or for detection of the re-emergence of a disease.

Critical success factors exist more at the level of infrastructure and the availability of reliable disease and vector information, rather than at the data collection and data quality level. Especially concerning vector-borne diseases, the limited links between epidemiological, ecological and entomological data have made it difficult to allow for the full capacity of an early warning system to function at its highest potential. This has been improving in the last decade in large part due to improving technology and the use of satellite imagery in veterinary epidemiology as shown in various papers(Purse *et al.*, 2004b), Brownstein *et al.*, 2005).

In conclusion, the success of a sentinel herd surveillance primarily depends on the purpose for which it was established, and more precisely on the actual establishment criteria regarding location, sentinel species and diagnostic methods. Detailed epidemiological knowledge of the agent under consideration is required to assure the adequate design of a sentinel surveillance programme, and is therefore the most critical success factor.

Acknowledgements

The authors would especially like to thank Dr. Audige, Dr. Iverson, Dr. McCluskey and Dr. Mumford for the information they provided in aid of this literature research. This paper was financially supported by the Swiss Federal Veterinary Office.

Table 3. 1 Examples of sentinel herd surveillance programs

Disease, condition	Country	Sentinel animal	Reference
Akabane disease	Saudi Arabia	Cattle,sheep, goat	(Abu Elzein *et al.*, 1998a)
Air pollution	Canada	Cattle	(Waldner *et al.*, 2001)
Avian Influenza	France, Holland	Birds	EUROPA IP/06/210
Bluetongue	Australia	Cattle	NAMP
Bovine dermatophilosis	USA	Cattle	(Hadrill and Walker, 1994)
Bovine viral diarrhoea virus	Canada	Cattle	(Waldner and Campbell, 2005)
East Coast Fever (Theileria)	Zambia	Cattle	(Billiouw *et al.*, 2005)
Epizootic Hemorrhagic disease	Sudan	Cattle	(Mohammed *et al.*, 1996)
Internal parasites	New Zealand	Deer	(Audige *et al.*, 1998)
Livestock comfort	USA	Cattle	(Cook *et al.*, 2005)
Lyme disease	USA	Dog	(Duncan *et al.*, 2005)
Rift Valley Fever	Africa	Sheep, goat	(Chevalier *et al.*, 2005)
St. Louis encephalitis	USA	Chicken	CDC
Trypanosomiasis	Burkina Faso	Cattle	(Paling *et al.*, 1987)
Vesicular Stomatitis	USA	Horse	(McCluskey *et al.*, 2002)
West Nile	USA	Crow	(Eidson *et al.*, 2001)
Western equine encephalomyelitis	USA	Chicken	CDC
Xenotransplantation	USA	Pig	(Iverson and Talbot, 1998)

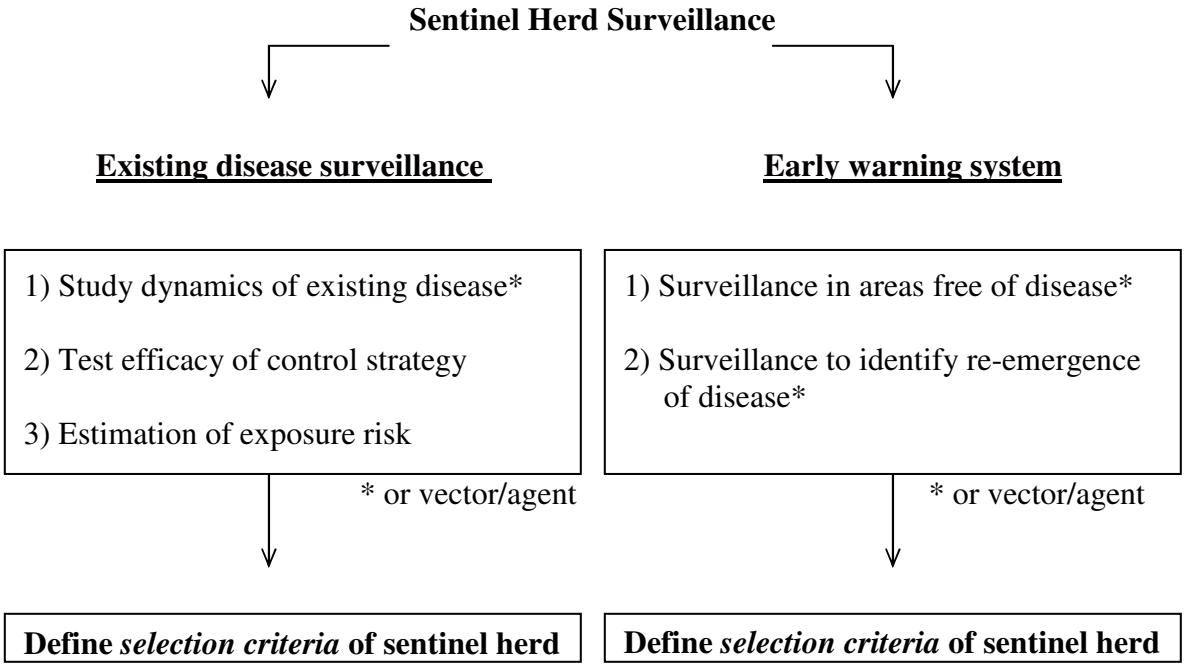

Fig.3.1 Diagram of sentinel herd surveillance design.

Taking into consideration the objectives, and subsequent sentinel herd selection.

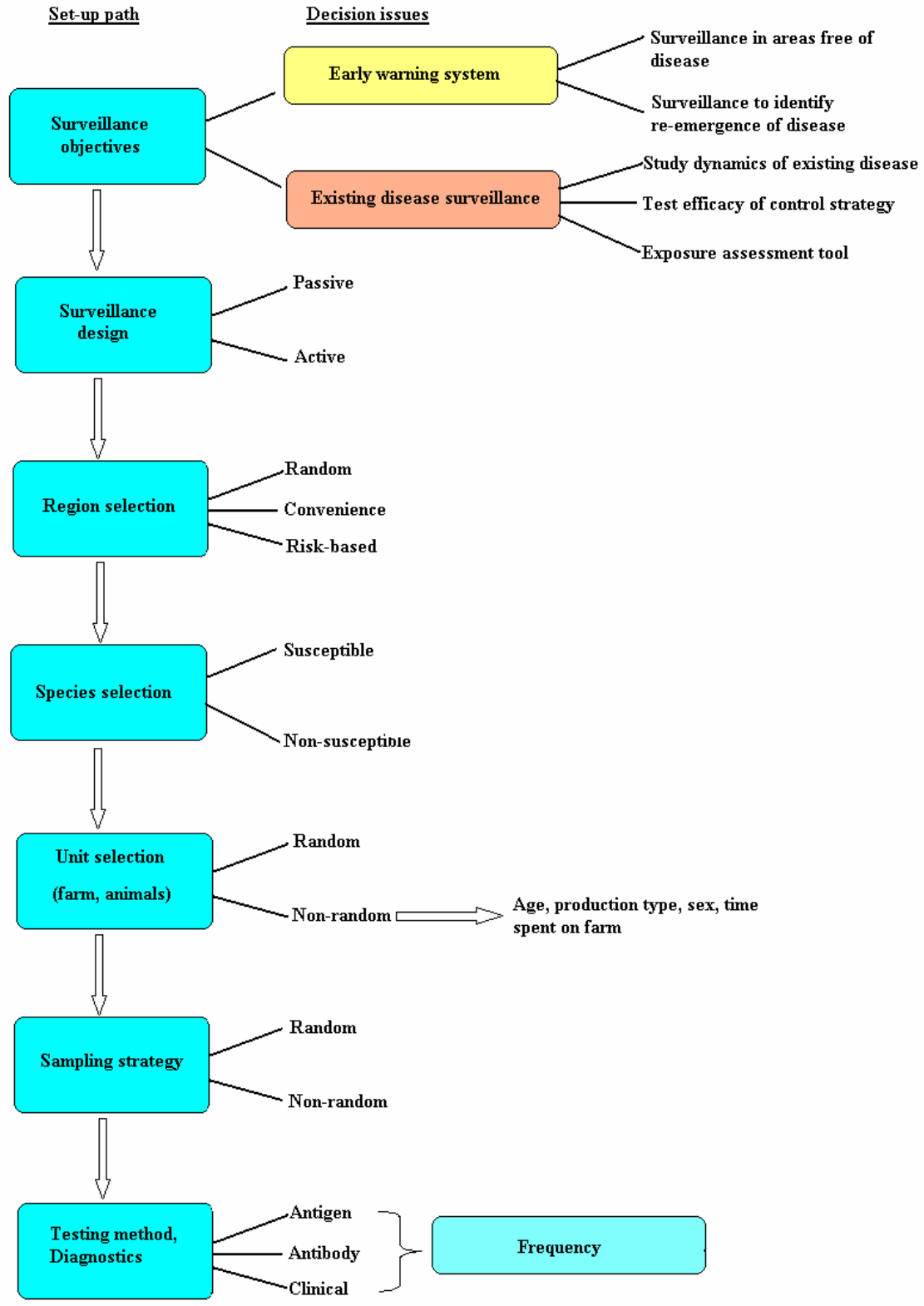

Figure 3.2. Set-up path for establishment of sentinel programme. Establishment of a sentinel herd programme through the definition of study objective, selection of sentinel herd site, sentinel animal species, sentinel unit selection, sampling strategy and selection of diagnostic procedure.

CHAPTER 4

Establishment of an early warning system against bluetongue virus in Switzerland

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This paper has been published in *Schweizer Archiv für Tierheilkunde* Band 148, Heft 11, November 2006, 593–598

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Abstract

Bluetongue (BT) is a vector-borne animal disease of economical importance due to the international trade restrictions likely to be put into place in a country once the infection is discovered. The presence of BT and its vectors in countries adjacent to Switzerland stresses the need of implementing a surveillance system and to raise disease awareness among potential stakeholders. A national survey in Switzerland 2003 indicated freedom of Bluetongue virus (BTV), although a single individual of the main BT vector *Culicoides imicola* was caught in the canton of Ticino. The survey also demonstrated that potential BT vectors, *C. obsoletus* and *C. pulicaris* are locally abundant in Switzerland. Therefore, a new surveillance method based on sentinel herds in high risk areas was implemented in 2004 for the early detection of both an incursion of BT vectors into Switzerland, and potential virus circulation among cattle.

Keywords: Bluetongue virus, Culicoides, sentinel herd surveillance, early warning system

Since the time of writing, dynamic changes in the current epidemic of Bluetongue in Europe have occurred. In the recent outbreaks in northern Europe (Belgium, France, Germany, and Holland), clinical symptoms have been seen in cattle, caused by Bluetongue virus serotype 8. This serotype has never been previously recorded in Europe. The vector responsible for outbreaks in southern Europe *(Culicoides imicola)* has not been detected in the affected regions mentioned as to date, but other Culicoides species were trapped (www.oie.int). In reaction to this development, two further sentinel farms located in the north of Switzerland will be added to the surveillance system.

Emergence and consequences of vector-borne diseases

Over the past few decades, vector-borne diseases have been emerging and spreading into areas previously free of such manifestations. Factors such as the shift in the style of husbandry management and the intensification of livestock production have contributed to the maintenance of the disease once it has occurred, but climate change has had the most profound effect on the capability of vector survival and establishment into new areas (Walther *et al.*, 2002). One of the major current epidemics involving at least 17 countries in the Mediterranean region is being caused by Bluetongue disease virus (BTV), and has caused the deaths of over 1 million sheep (Purse *et al.*, 2005). BT entered Europe via two paths. The first detection of the disease originated from the Greek islands in 1998 and consequently spread northward into the Balkan area and westward onto the French and Italian islands of Corsica, Sicily and Sardinia, respectively. This was followed in 2000 by an incursion from Tunisia and Algeria and spread onto mainland Italy (Tuscan area) and the Spanish Balearic islands. The second detected movement started in Morocco in 2004 and spread northward into Spain and Portugal. The latter two countries are still reporting cases at present (www.oie.int).

Switzerland shares borders with mainland France, where *Culicoides imicola* has been found, as well as with Italy where the disease and its vector are present (Breard *et al.*, 2004, De Liberato *et al.*, 2005). BT disease occurs exclusively where its competent vector is present and in terms of the European epidemic, the main insects responsible for the spread of this disease are *C. imicola.* It has been recently determined that *C. obsoletus* and *C. pulicaris* are also considered as potential vectors (Caracappa *et al.*, 2003, Savini *et al.*, 2005). All three of these species have been found in Switzerland (Cagienard *et al.*, 2006a). The risk of BT spreading into Switzerland was attributable to its vicinity to Italian BT outbreak locations (last outbreak was 360 km from the Swiss border) as well as the threat from the northern European outbreaks, and to the fact that central Europe is experiencing a temperature rise much higher than the global trend (www.umwelt-schweiz.ch). This could allow vectors to find new suitable habitats or cause a shift in vector competence. Although the Alps form an important natural barrier for the further spread of the vectors, the southern and western part of Switzerland could be affected by windborne vectors originating from Italy, Germany and France, respectively.

Bluetongue disease

Bluetongue disease is a non-contagious, infectious insect-transmitted viral disease that affects domestic and wild ruminants (Purse *et al.*, 2005). The virus belongs to the genus Orbivirus in the Reoviridae family with 24 serotypes occurring worldwide. Although the virus replicates in most, if not all ruminant species, severe disease only occurs in certain breeds of sheep. Cattle, however, play an important role as amplifying hosts since insect vectors such as *C. imicola* have been shown to prefer them as opposed to sheep (Nevill, 1978), and they can remain viremic for approximately 60 days post infection. Because cattle do not develop clinical signs, there is a risk that the virus is maintained in silent circulation. However, cold weather limits the over-wintering capacity of vectors whereby susceptible hosts would not be affected by these potential viremic hosts. Symptoms in sheep vary from fever, hyperemia and oedema possibly leading to cyanosis although the actual clinical sign of a blue tongue is rarely seen. Infection of pregnant ewes may lead to abortions as well as mummified, stillborn or weak lambs (www.defra.gov.uk).

Although there are 24 recognized BTV serotypes occurring worldwide, the current epidemic in Europe involved serotypes -1, -2, -4, -9 and -16. BTV -1, -4, -9 and -16 have entered Europe from the east, whilst another path from the south introduced BTV -2 and -4. Each serotype is usually linked to a certain geographic region in the world, for example, BTV-1 has been isolated in India, and BTV-2 usually occurs in South Africa and the United States, where BTV-10, -11, -13 and -17 also occur. Australian cases are caused by BTV-1, -3, -9, -15, -16, -20, -21 and -23 serotypes. BTV -1-16, -18, -19 and -24 are responsible for African outbreaks whilst BTV -1, -3, -4, -6,-8, -12 and -17 circulate in the Central American-Caribbean Basin (www.fas.org). The effect and severity of disease on the individual host depends on the BTV serotype, the vector species, as well as the actual breed of animal host involved.

Bluetongue vectors

BT is exclusively related to the presence of the virus in the competent vector: insects of the Culicoides species. In concurrence with BTV serotypes, a large number of Culicoides midges are responsible for the spread of the disease in different countries. The principle vectors in

Australia are *C. wadai, C. brevitarsis, C. fulvus* and *C. actoni* midges while in the United States, *C. sonorensis* is the main vector (www.fas.org). The midge species of *C. imicola,* also responsible for Asian and African BT outbreaks, has established itself in Europe and is considered the main cause of the current European epidemic. The presence of different *Culicoides* midges in various countries is due to the specificity of habitat preference displayed by each vector (Kline and Wood, 1988, Schmidtmann *et al.*, 2000). The peculiarity of the European epidemic brings to light the ability of certain insects to develop a vector competent status as is the case with *C. obsoletus* and *C. pulicaris*. BTV serotypes 2 and 9 have been isolated from wild-caught *C. obsoletus* (De Liberato et al., 2003, Savini et al., 2005) and *C. pulicaris* (Caracappa *et al.*, 2003). Previous and ongoing surveillance has demonstrated a high abundance of *C. obsoletus* in certain regions of Switzerland followed by a considerable number of *C. pulicaris*, among other *Culicoides* spp. (Cagienard *et al.*, 2006a).

Surveillance in Switzerland

In order to monitor rare or exotic diseases such as Bluetongue disease or other vector-borne diseases i.e. African Horse sickness (AHS) which is also transmitted by Culicoides spp, it is important to achieve and maintain sufficient disease awareness by informing all potential stakeholders. Pamphlets describing BT disease have already been distributed by the Swiss Federal Veterinary Office (www.bvet.admin.ch) and surveillance has been implemented since 2002 (Cagienard *et al.*, 2006b). Presently, in the Swiss Ordinance of Epizootics, BT is listed in the highly contagious disease section. In addition to the current surveillance, veterinarians should be aware of the typical symptoms prevailing in sheep, although clinical diagnosis of BT in cattle is difficult. Increased vigilance is required especially during and shortly after the vector season (June-September). Upon the suspicion of BT symptoms, the respective cantonal veterinarian should be immediately alerted, and further investigations will consequently follow.

Surveillance system implemented for Bluetongue monitoring

The aim of initiating BT surveillance was to create an early warning system to detect the primary incursion of the agent and/or vector before substantial spread may occur. The current

surveillance system in Switzerland evolved from an initial baseline surveillance phase using random sampling into an early warning phase using risk-based sampling in established sentinel herds. The main methods used consist of entomological surveillance (vector trapping) and serological surveillance of hosts. The initial objectives of BT surveillance were to establish the status of Switzerland with respect to vector presence and host immune status. The current focus is on the early detection of changes in vector populations and/or host status. The evolution of the existing surveillance is explained in more detail below.

Initial baseline surveillance

To determine the immune status of Swiss cattle, a nationwide serological survey was conducted in 2003, involving 660 randomly selected cattle farms. Cattle were chosen as the target species primarily since they act as the reservoir species for the BTV in sheep. They also present an earlier antibody response post-infection (www.oie.int), cattle farms are more abundant and more widely distributed in Switzerland and, finally, it has been shown that *Culicoides* midges have a feeding preference on cattle compared to sheep (Nevill, 1978). High-risk locations based on vector habitat requirements were defined as areas with an average annual temperature ≥12.5°C as well as an annual average humidity > 60%. In addition to the main climatic factors, host number and geographic location were also taken into account. The farm should have > 3 cattle present which remain at an altitude of <1100 meters above sea level during the summer period. The serological survey confirmed the freedom-of-BTV-infection status of Switzerland according to international standards. The entomological part of early surveillance consisted of 41 trapping sites distributed in high-risk areas for the occurrence of Culicoides. These sights were sampled in July and September, 2003. Regarding vectors, *C. obsoletus* and *C. pulicaris* were found to be abundant in many locations but only 1 single specimen of the main vector *C. imicola* was caught (Cagienard *et al.*, 2006a).

Early warning system based on sentinel herds

The monitoring of sentinel herds was shown to be an effective method for the surveillance of vector-borne diseases including BT in other countries such as Saudi Arabia, China and

Australia (Ward *et al.*, 1995, Abu Elzein *et al.*, 1998a, Kirkland *et al.*, 2002). A sentinel herd is defined as a cohort of animals at a pre-determined location, which is monitored over a specified period of time with respect to a specified disease agent (Ward *et al.*, 1995). Sentinel herds are usually carefully selected to be located in high-risk areas of disease occurrence or likely vector establishment. Such an approach is categorized as a targeted surveillance system as opposed to random surveillance (Stark *et al.*, 2006). This is an important difference in order to increase the probability of detection of any first incursion of disease at the earliest time possible.

Serological surveillance of sentinel herds

The sentinel herds used in BT surveillance in Switzerland were selected based on an assessment of risk factors and the experience made in other countries. The most influential factors were location in terms of altitude and climatic conditions, occurrence of vector competent species as well as host species. The establishment of sentinel farms as an early warning system for *C. imicola* introduction and BTV infection was established in 2004, modified in 2005 and will continue to run in the future. The aim is to determine the immune status of the sentinel herds regarding BTV. Sentinel herds are also used as trapping sites to monitor the frequency and distribution of the BTV vectors in Switzerland. Using the same criteria as in the baseline survey, sentinel farms were selected in locations considered at risk in Switzerland (Fig. 4.1). Cattle were the sentinel species of interest. Additionally, sentinel locations were included if the farm had a limited or absent utilization of insecticides on the farm. Serum samples are annually collected in November and sent to the Institute of Virology and Immunoprophylaxis for diagnostic procedures. Given the fact that Switzerland is presently BTV-free, this study will allow for the determination of incidence rates of new infections in the sentinel herds.

Entomological surveillance

The entomological part of the surveillance involves setting up Onderstepoort black light traps (Venter *et al.*, 1997) at the selected sites in high risk areas of Switzerland, focusing on the canton of Ticino and collecting samples during the months of June to September. Due to its neighboring location to Italy and its specific micro-climate, Ticino was considered as a high-risk

zone for both the occurrence of BTV and its vectors. A total of 7 trapping locations (Fig. 4.2) were used in 2005 and samples were first microscopically analysed according to the protocol established by the Institute of Animal Health (IAH) to determine the frequency of Culicoides spp, especially *C. imicola, C. obsoletus* and *C. pulicaris* if present. Samples were also analysed by PCR to confirm absence of *C. imicola* (Cetre-Sossah *et al.*, 2004). In addition to the sentinel herds described above for serological surveillance, horse stables and goat farms were also chosen as trapping locations within high-risk areas since it has been demonstrated that BTV vectors are recorded in high numbers near these hosts (Racloz, unpublished data).

Results 2004-2005

Results of the entomological samples collected in 2004 and 2005 confirm the abundance of the *C. obsoletus* species as well as the variety of different Culicoides spp. caught in the light traps during a single night. The main vector for BTV transmission, *C. imicola*, has not been not detected since 2003 (Fig. 4.3). The serological sampling of sentinel herds has confirmed the infection-free status of Swiss cattle for BTV. It can therefore be concluded that Switzerland is currently a BTV-free country.

Conclusions

The occurrence of BT is dependent on vector habitat, host preference and vector competency. It is thus possible to determine locations considered to be at risk of disease occurrence. In the case of the current entomological surveillance, the biology of *Culicoides* spp. has been taken into consideration when determining risk locations. Only a few sites fulfill all selection criteria. They are all included in the current surveillance system. Therefore, the specific trapping sites currently used allow for the recording of changes in vector abundance and species composition. Switzerland has recorded one specimen of *C. imicola* in 2003. The fact that it has been the only case so far in the two years of surveillance that followed combined with the unsuitability of over-wintering conditions, limit the potential of BT disease being spread by this vector. Countries where BT outbreaks have occurred in the absence of *C. imicola* are

investigating the potential role of *C. obsoletus* and *C. pulicaris* and their capability of spreading the virus efficiently. Therefore, a targeted entomological and serological surveillance needs to continue in order to monitor the presence of the virus and these latter vectors which seem to be adapted to the climatic and environmental conditions of certain regions in Switzerland.

Finally, this early warning system will not only serve for BT surveillance, but also as a model of surveillance for other vector borne diseases such as anaplasmosis and babesiosis, two tick-borne diseases. The fact that the main vector of these agents, *Ixodes ricinus*, is commonly found in Switzerland, combined with the recent outbreak of anaplasmosis in 2002 in a single farm (Gern and Brossard, 1986, Brulisauer *et al.*, 2004, Hofmann-Lehmann *et al.*, 2004), and the presence of Babesia spp in Switzerland , support the usefulness of an early warning system to monitor the occurrence of these diseases.

Acknowledgements

The authors would like to thank the farmers involved for their cooperation, as well as Simona Casati, Sarah Albini and Daniela Huessy for their help with the entomological field work and serological diagnostics respectively.

Figure 4.1: Location of sentinel farms for serological surveillance (farms shown as ■).

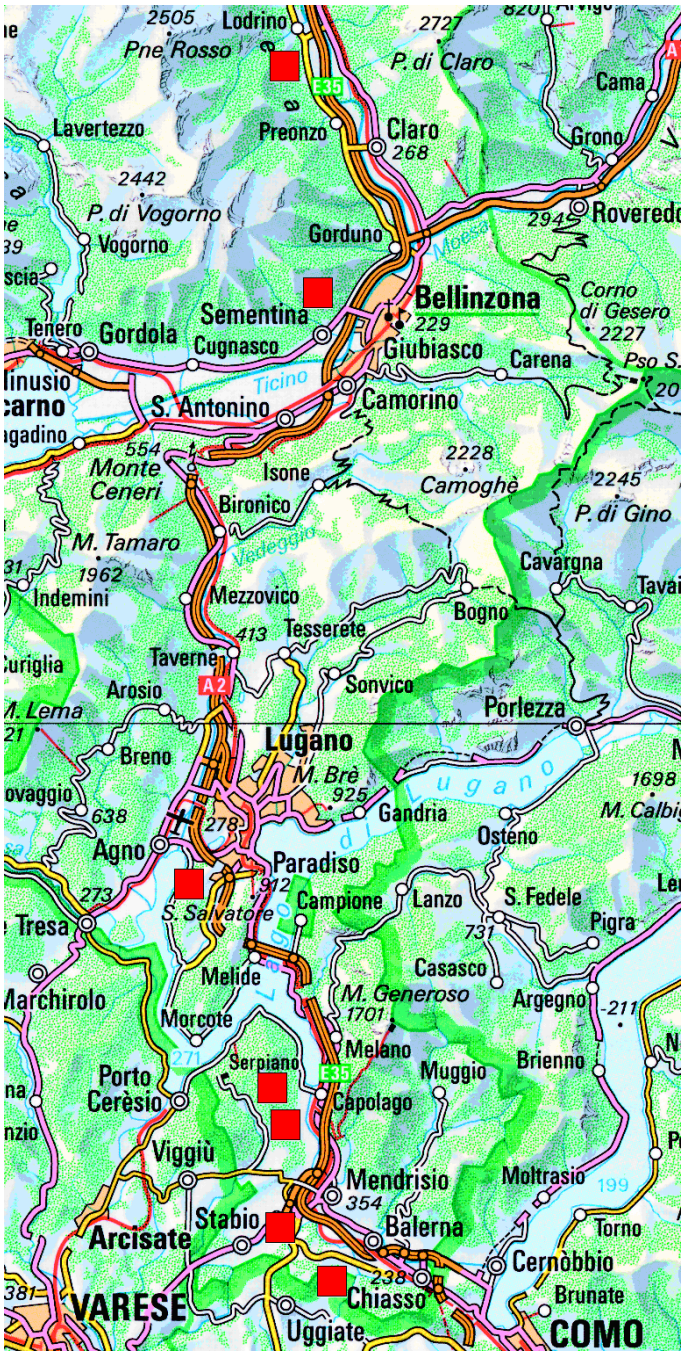

Figure 4.2: Location of Culicoides trapping sites in the canton of Ticino, 2005. (Farms shown as ■)

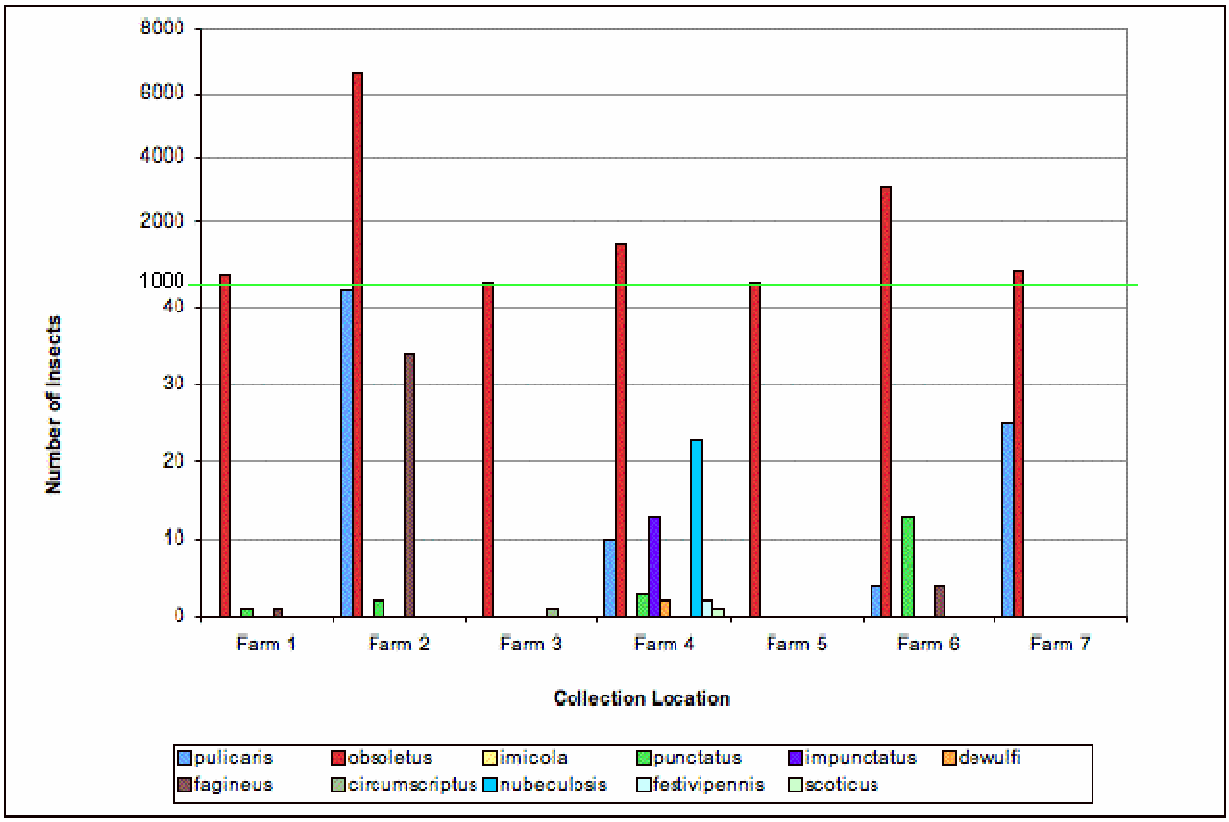

Figure 4.3: Number of Culicoides species collected in a single night catch in Ticino, Switzerland - June 2005.

CHAPTER 5

Unpublished results of sentinel herd serological blood sampling

Choice of sentinel herds

The selection of sentinel sites for BT in Switzerland was based on altitude, climatic conditions, and presence of competent vector species as well as host species. Areas having an average annual temperature ≥12.5°C, an annual average humidity > 60%, more than 3 sentinel animals at an altitude of <1100 meters above sea level in summer, and farms where no insecticides used were chosen. In the canton of Ticino, due to the host preference shown by *Culicoides*, it was decided to also include two equine centers and a goat farm.

These farms were also used to test for anaplamsa and babesia due to the wide distribution of ticks, and farmers' observations, although the testing period would only allow the detection of antibodies due to the fact that the highest peak of tick activity is generally earlier in June.

Sampling strategy

Serological blood sampling was carried out on 12 cattle farms in 2005, of which 7 of those were also part of the entomological surveillance. The latter farms were located in the canton of Ticino. The remaining five farms were located in the cantons of Fribourg, Neuchâtel, Vaud and Grisons. In 2006, the same farms were kept with the addition of two farms in the canton of Basel, where both serological and entomological surveillance took place. Finally in 2007, a further entomological trap was placed in the canton of Zurich.

The blood sampling took place from November-January after vector season. This would allow for any infections that might have occurred to be clearly present in the diagnostic tests used, as well as allowing a long exposure period for potential vector attack. Blood was taken from the cattle tail vein, and sent to the corresponding labs for diagnostics. Cattle were chosen as serological sentinel animals due to the recorded host preferences which *Culicoides* midges present (Nevill, 1978), as well as being more abundant and accessible in Switzerland.

Anaplasma marginale detection was carried out in the Center for zoonoses, bacterial animal diseases and antibiotic resistance (ZOBA) in Bern using a competitive enzyme linked immunosorbent assay (C-ELISA) for general *Anaplasma* species. The Institute of Parasitology, in Zurich tested for *Babesia divergens* using and ELISA test. Finally BT blood samples were sent to the Institute of Virology and Immunoprophylaxis in Bern for testing with a C-ELISA. If any of these tests were positive, a PCR was then carried out with the exception of BT where a retest would take place.

Entomological sampling took place on a twice per month basis for the months of June-October, as discussed in Chapter 7.

Results

In total, 275 cattle were tested in 2005 and 2006 on 12 and 14 herds respectively. From these, none were positive for bluetongue disease, yet 4 and 2 presented positive Anaplamsa and Babesia results respectively, in the first round of diagnostic tests. Due to the sensitivity and specificity of the tests used, these were repeated in a second round of tests and some retained an ambiguous status, especially originating from farms located in western Switzerland.

Discussion

The reason why the tick-borne diseases were more difficult to diagnose properly is since many cross reactions were possible during the initial diagnostic process as well as the quality of the samples tested. As mentioned, testing was carried out in November, which is far from the acute phase of potential infection. Further tests were carried out in ZOBA to determine whether it was another Anaplasma species, in specific *A. phagocytophilum*, a zoonotic disease. After additional testing carried out by Dr Albini, which proved that it was not the latter species, it was decided not to pursue with the procedure.

CHAPTER 6

Use of mapping and statistical modeling for the prediction of bluetongue occurrence in Switzerland based on vector biology

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This paper has been published in *Veterinaria Italiana*, 43 (3), 513-518,
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Abstract

Due to the spread of bluetongue (BT) in Europe in the last decade, a sentinel surveillance programme was initiated for Switzerland in 2003, consisting of serological sampling of sentinel cattle tested for BT virus antibodies, as well as entomological trapping of *Culicoides* midges from June until October.

The aim of this study was to create a 'suitability map' of Switzerland, indicating areas of potential disease occurrence based on the biological parameters of *Obsoletus* Complex habitat. Data on *Culicoides* catches from insect traps together with various environmental parameters were recorded and analysed. A multiple regression analysis was performed to determine correlation between the environmental conditions and vector abundance. Meteorological data were collected from 50 geo-referenced weather stations across Switzerland and maps of temperature, precipitation and altitude were created. A range of values of temperature, precipitation and altitude influencing vector biology were obtained from the literature. The final combined map highlighted areas in Switzerland which are most suitable for vector presence, hence implying a higher probability of disease occurrence given the presence of susceptible animals. The results confirmed the need for an early warning system for the surveillance of BT disease and its vectors in Switzerland.

Keywords : Bluetongue, *Culicoides*, Geographic Information System, map, sentinel surveillance, vector biology.

Introduction

Since 2003, Switzerland has put in place serological and entomological plans in order to detect and monitor the presence/absence of BTV by the periodical testing of sentinel animals and to evaluate the risk linked to the presence and abundance of *Culicoides* species (Cagienard *et al.*, 2006b). To date, there has been no evidence of the virus although high numbers of *Culicoides* midges have been recorded (Cagienard, 2004). The monitoring of sentinel herds has shown to be an effective method for the surveillance of vector-borne diseases including BT in other countries such as Saudi Arabia, China and Australia (Ward *et al.*, 1995, Abu Elzein *et al.*, 1998a, Kirkland *et al.*, 2002). However, sentinel herds need to be carefully selected and located in high-risk areas of disease occurrence or in suitable habitat of the vectors (Racloz *et al.*, 2006a). The selection of sentinel sites for BT in Switzerland was based on altitude, climatic conditions, and presence of competent vector species as well as host species. Areas having an average annual temperature $\geq$12.5°C, an annual average humidity > 60%, more than 3 sentinel animals at an altitude of <1100 meters above sea level in summer, and farms where no insecticides were used, were classified as 'High-risk locations' (Cagienard *et al.*, 2006b). Originally, a total of 12 sentinel cattle herds were chosen for serological monitoring in 6 different administrative regions (cantons) of Switzerland and 7 farm locations were selected for entomological trapping in areas considered at risk for the presence of the vectors (Racloz *et al.*, 2006b). The aim of this study was to integrate data collected from the entomological trapping sites as well as climate data into a Geographic Information System (GIS) in order to create a map indicating areas of potential BT disease occurrence in Switzerland based on the biological parameters of *Culicoides* habitat. These parameters form part of the 'environmental envelope' of the vector, a term similar to 'climatic suitability envelope' (Tatem *et al.*, 2006). This concept defines how key climatic and environmental factors form a niche which is occupied by specific species.

The outputs generated through the use of mapping techniques were used to evaluate the positioning of the sentinel herds. Similar techniques have been used in other countries to optimize BT surveillance (Conte, 2004) or other diseases such as Malaria (Hassan *et al.*, 2003).

Material and methods

Entomological data were collected using Onderstepoort blacklight traps in 7 sampling sites for the years 2005 and 2006. Minimum and maximum temperature during trapping, insect abundance and diversity, host species present and altitude for each trap location were recorded. Depending on availability (monthly or yearly averages), climate data (wind speed, relative humidity, temperature and precipitation) were obtained from the Swiss Meteorological Office.

Multiple regression analysis was carried out in order to determine the effect of these parameters on vector abundance, which ranged from 1 to 15,664 insects per trapping night. The independent variables included in the analysis were: altitude of farm location, minimum and maximum temperature on trapping site, host species present (based on the number of cattle, sheep or goats present on farm), relative humidity, wind speed and precipitation. A negative binomial regression analysis was carried out with the objective of using its coefficients as weights in the Spatial Process Model to map areas of increased vector suitability. Since the trapping sites were initially chosen in 'high risk locations' with similar climatic, host density and geographical features, it was difficult to show any significance in the other parameters tested. Hence only 3 from the total factors were used to create the suitability maps. It would be useful to trap in areas with varying altitudes and temperature limits.

Thematic maps for altitude, precipitation and annual average temperature were created by using ArcGis (Version 8.3 , Environmental Systems Research Institute, Inc.) through the input of all 50 meteorological stations in Switzerland. Smoothing was performed by ordinary kiriging, set to incorporate three points, with the exception of the altitude map, which originated from an existing elevation model. Suitability categories (Table 6.1) were created based on input values obtained by literature review of Obsoletus Complex data (Mellor *et al.*, 2000, De Liberato *et al.*, 2005, Purse *et al.*, 2005, Carpenter *et al.*, 2006, Osmani *et al.*, 2006, Purse, 2006). These ranges of values were divided into 4 classes indicating low to high vector suitability for each factor. The limits for the classes used ranged from the minimum to the maximum values found in the literature (environmental envelope). Through the map calculator function of ArcGIS, the maps were layered and categories added together to create a final vector suitability map. This map (Fig. 6.4) indicates areas of potential BT occurrence based on Obsoletus Complex vector biology for Switzerland.

Results

Through the analysis of entomological samples (one sample corresponded to a one-night trap collection) from 7 different farms, a total number of 27,256 *Culicoides* were identified for 2005 (62 samples collected), and 43,527 in 2006 (42 samples collected), of which the Obsoletus Complex accounted for 96% and 98% of the catches respectively.

The preliminary statistical analysis showed that maximum temperature had the most significant effect on vector abundance with a rise of 1ºC bringing an increase of 18% in vector abundance. However due to the lack of significance in the other independent variables, only three parameters were kept and were assigned equal weights ([altitude] + [temperature] + [precipitation]) for the creation of the combined map.

In Figures 6.1, 6.2, 6.3 and 6.4 suitability maps are reported. The blue class indicates all areas most unsuitable for vector presence whereas the red class identifies areas most suitable for Obsoletus Complex presence.

Discussion

Until now, the occurrence of BT has been linked to areas where the vector is present and where appropriate climatic and environmental conditions are available. Through the review of literature, it was possible to collect values for parameters affecting vector biology and to analyze them through GIS technique. This allows to determine areas at risk of disease occurrence. The term 'environmental envelope' has been well described in Purse et al. (Purse, 2006), and specifies that the environmental envelope for non – *C. imicola* is quite distinct from its old world counterpart since they seem to occupy cooler and wetter regions as compared to traditional *C. imicola* ranges. This is supported by the maps generated in this study, along with actual data from trapping surveillance in Switzerland.

Since trapping sites were chosen to include suitable locations for *Culicoides* presence, it has not been possible to quantify and evaluate the real association between environmental and climatic factors as well as vector abundance. Sites where catches have been made were very similar in altitude, temperature and precipitation and this didn't allow to find a statistical significance with the numbers of *Obsoletus* Complex midges that were collected. A further

limitation was that values for precipitation did not differentiate between snow and rainfall. This may have caused biased results with a consequent overestimation of suitable areas since snow is considered an unfavorable factor for vector development. This could be corrected by limiting climate data to the vector seasons. Nevertheless, the risk areas in the final map are in agreement with the initial choice of sentinel locations used in the surveillance program (which was established prior to creation of maps) although it indicated an unexpected high vector suitability in the North of the country. Due to this outcome and in combination with the current epidemic in northern Europe, two further sentinel herds were added in northern Switzerland, making a total of 14 sentinel cattle herds in the serological monitoring surveillance system, 9 of which are also monitored entomologically.

The recent spread of bluetongue in Northern Europe, area in which *C. imicola* has never been reported, confirm the important role of novel vectors in the transmission of the disease. High abundance of Obsoletus Complex as well as a large variety of other *Culicoides* species recorded in Switzerland are comparable to values recorded in other European countries affected by Bluetongue (De Liberato *et al.*, 2005, Savini *et al.*, 2005). Through the combined use of biological and spatial information, it is possible to determine areas which are more suitable for vector presence, hence at a higher risk for BT occurrence.

After completion of data collection planned for 2007, suitability maps will be considered a useful instrument to guide the assessment of an extensive surveillance program in terms of a targeted sampling strategy, as well as reinforcing its role as a tool in supporting existing surveillance programs or by helping in prospective decision making processes, for example in other vector-borne diseases.

Acknowledgments

The authors would like to thank M. Kuhn, B. Häsler, C. Bill and A. Cagienard for their help, and the Swiss Meteorological Office for providing climate data.
This study was funded by the Swiss Federal Veterinary Office.

Table 6.1: Table of suitability classes used for altitude, temperature and precipitation based on values found in literature- influencing the vector biology

Parameter	Suitability Scale			
	High	**Medium High**	**Medium Low**	**Low**
Altitude (meters)	488-1261	10-488	-	<9 and >1261
Annual average temperature(°C)	>12	10-12	8-10	<8
Annual precipitation (mm)	815-1224	483-641	>1224	<815

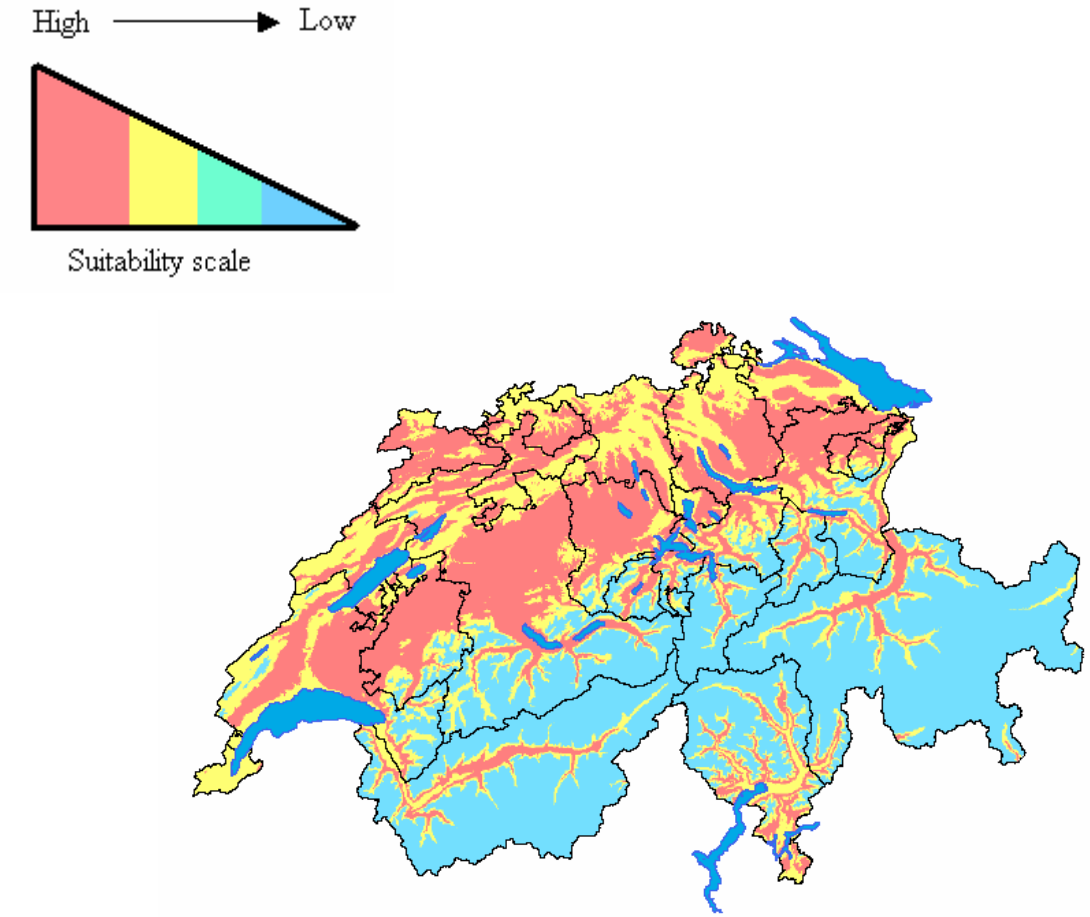

Fig. 6.1 Altitude Suitability Map of Switzerland.

Map highlighting areas suitable for *Obsoletus* Complex habitat according to altitude.

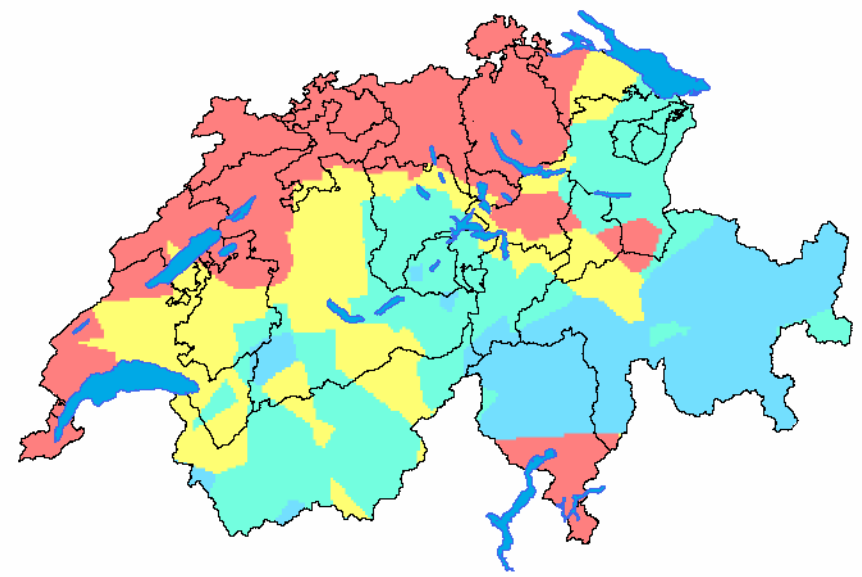

Fig. 6.2 Temperature Suitability Map of Switzerland.

Map highlighting areas suitable for *Obsoletus* Complex habitat according to temperature.

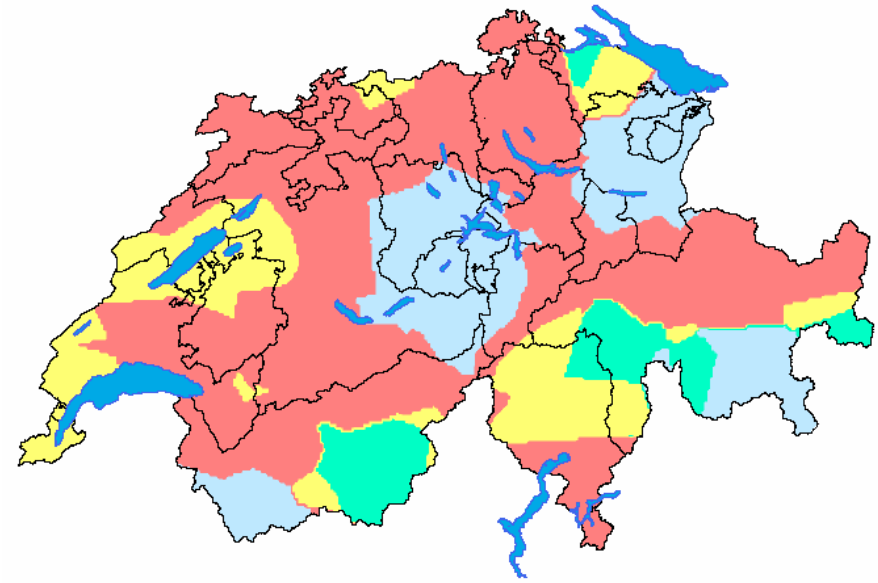

Figure 6.3 Precipitation Suitability Map of Switzerland.

Map highlighting areas suitable for *Obsoletus* Complex habitat according to precipitation

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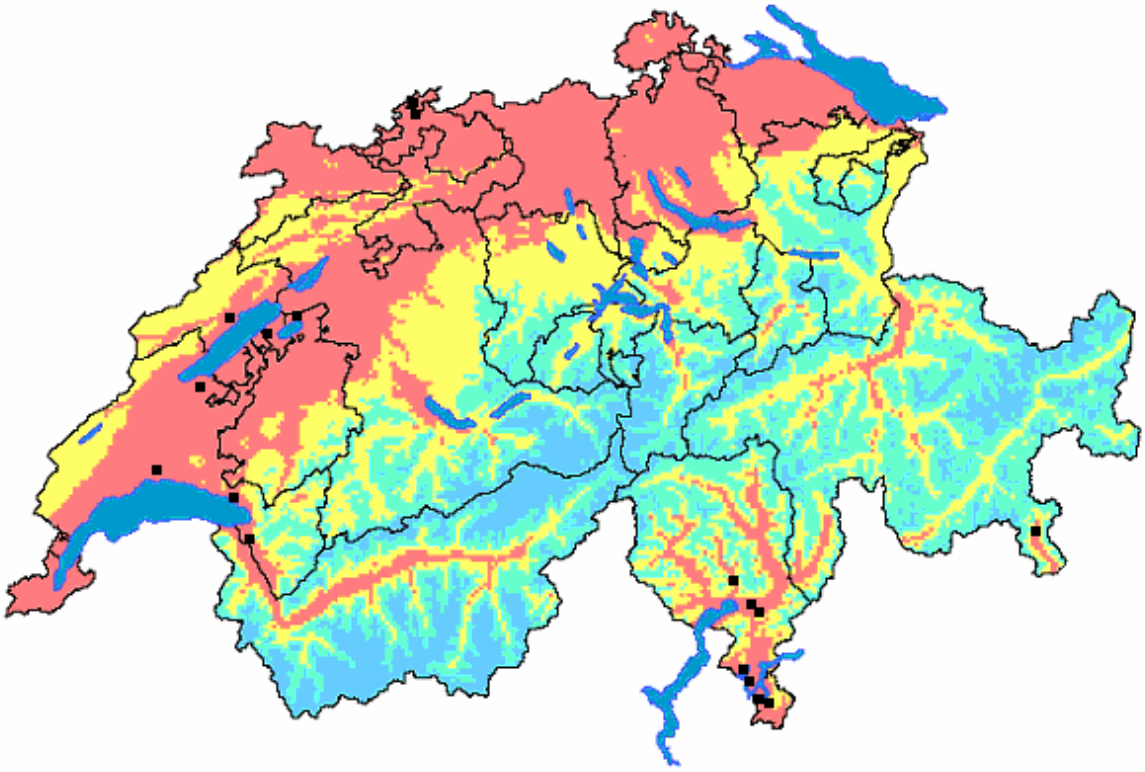

Figure 6.4 Final combination map.

Obsoletus Complex suitability map based on temperature, altitude and precipitation values. (Current sentinel herd locations are marked as ▪).

CHAPTER 7

An investigation on the *Culicoides* species composition at seven sites in southern Switzerland

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This paper has been accepted in the journal of Medical and Veterinary Entomology

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Abstract

In the past decade, there have been regular outbreaks of bluetongue (BT) in many parts of Europe. Due to the presence of BT disease and its vectors in countries adjacent to Switzerland, an initial entomological survey was conducted in 2003, which established the presence of several midges of the genus *Culicoides* (Diptera: Ceratopogonidae). Subsequently, a sentinel herd monitoring system was established with the primary entomological aim being the determination and further study of *Culicoides* population compositions.

Insects were collected in 2005 and 2006 at seven sentinel herd sites in the south of Switzerland (canton of Ticino) near the border of Italy, using Onderstepoort-type light traps. This region is botanically and zoologically similar to the Mediterranean and is one of the warmest and most humid areas of the country, hence it is considered a potential access path for BT disease into Switzerland. Collections were made at 4 cattle farms, two equestrian centers and one goat farm. Sites were sampled four times per month from June to October. Traps were operated from dusk until dawn and samples were collected monthly for analysis through microscopy as well as a *Culicoides imicola*-specific PCR.

Results confirmed the absence of *C. imicola* (Kieffer) and demonstrated that the potential BTV vectors are highly abundant, notably: *Culicoides obsoletus* (Meigen), *Culicoides scoticus* (Downes & Kettle) and *Culicoides dewulfi* (Goetghebuer) *subgenus Avaritia* and *Culicoides pulicaris* (Linnaeus) *subgenus Culicoides*. These findings expand the current knowledge of *Culicoides* population composition in the southern part of the Switzerland. *Culicoides cataneii* (Clastrier), *Culicoides flavipulicaris* (Dzhafarov), *Culicoides indistinctus* (Khalaf), *Culicoides nubeculosus* (Meigen) and species of the *Grisescens* complex were reported for the first time in Switzerland.

Key words. *Culicoides*, Switzerland, bluetongue disease, sentinel herd surveillance

Introduction

Since 1998, there have been regular outbreaks of bluetongue (BT) in many parts of Europe, spreading northwards into southern Italy, Spain and France via one pathway, as well as westwards into the Balkan area in a second pathway (Purse *et al*., 2005). The disease is caused by the BT virus, an arbovirus (*Orbivirus*, *Reoviridae*), affecting domestic and wild ruminants (Taylor, 1986). The virus is transmitted by Diptera in the family Ceratopogonidae, belonging to the genus *Culicoides* (Mellor *et al.,* 2000). The main vector responsible for the incursion into the Mediterranean region is the Old World species, *Culicoides imicola* (Kieffer), yet to date there have been other vectors implicated in the recent Northern European outbreaks of 2006 as well as in countries such as Bulgaria and Italy in areas where *C. imicola* were found to be absent (Purse *et al.*, 2006a). An entomological survey conducted in Switzerland in 2003, not only indicated the presence of several suspected vectors, belonging to the *Obsoletus* and *Pulicaris* group, but also a single specimen of *C. imicola* in the southern part of the country (canton of Ticino) (Cagienard *et al*., 2006). The finding of a single specimen of *C. imicola* was exceptional and the epidemiological impact of this species in Switzerland is to be considered as negligible. Each *Culicoides* species or group has a unique geographical and ecological niche, although some overlapping occurs, as well as varying capacities to transmit different BTV serotypes of which there are presently 24 known worldwide (Purse et al., 2005). Current evidence indicates that the BT virus serotype 8 (BTV 8) causing the recent outbreak in northern Europe was spread by *Culicoides* midges belonging to the *Obsoletus* group (Mehlhorn *et al.*, 2007). Previous outbreaks in southern Europe (BTV 1,2,4,6,9 and 16), in some regions of Italy and Bulgaria, showed species from the *Obsoletus* group (only in Italy) and *Culicoides pulicaris* (Linnaeus) to be the predominant potential vectors (Caracappa *et al.*, 2003; Conte *et al.*, 2003; De Liberato *et al.*, 2005; Purse *et al.*, 2006b).

Due to the presence of BT and potential *Culicoides* vectors in countries adjacent to Switzerland, a detailed knowledge of the *Culicoides* population composition is required to estimate the risk of introduction into and spread of BTV within the country. The geographical location of the Canton Ticino, compared to the rest of the Switzerland, is of interest since it is located south of the Alps bordering Italy. This region is botanically and zoologically similar to the Mediterranean, and is one of the warmest and most humid areas of the country, hence it is

considered a potential access path for BT disease into Switzerland. In fact, considering the recent climatic changes, others new vectors have been reported in this particular region of Switzerland: *Rhipicephalus sanguineus* (Latreille, a tick of African origin) and *Aedes albopictus* (Skuse, the Asian Tiger Mosquito) (Bernasconi *et al.*, 2002; Flacio *et al.*, 2004).

Hence, a sentinel surveillance program was established in Ticino with the primary entomological aim being the determination and further study of *Culicoides* population compositions.

Materials and Methods

Collection sites

Using Onderstepoort-type light traps (Paweska *et al.*, 2003), insects were collected at seven sentinel herds (Table 7.1 and Fig. 7.1) in the Ticino canton, near the border of Italy, in the south of Switzerland (Racloz *et al.*, 2006b). Farms with cattle, horses and goats were targeted due to their potential of attracting BTV vectors. The majority of traps though were located on cattle farms since they are more abundant and widely distributed in Switzerland, and it has also been shown that *Culicoides* midges have a feeding preference for cattle compared to sheep (Nevill, 1978). Farms were chosen to fulfill the following criteria: an average annual temperature ≥12.5°C, an annual average humidity >60%, be located below 1100 meters above sea level, and having >3 cattle remaining on farm during the summer months as opposed to being brought to alpine pastures (Racloz *et al.*, 2006a).

Insects were collected for two successive nights twice per month. The data presented here derived from the collections made from June-October during 2005 and 2006. Insects were collected and stored in 70% alcohol. The identification of *Culicoides* was conducted using three approaches. The majority of samples were analysed for vector abundance data, and the remaining samples were split into two, firstly for a more detailed identification process, and secondly for the determination of *C. imicola* presence. Of 238 collections made 109 samples were analysed at the Federal Veterinary Office to determine the frequency and abundance of *Culicoides* species, whilst 80 samples were examined for a more in-depth identification procedure. A total of 49 samples were analyzed with PCR to determine the presence/absence of *C. imicola.*

Entomological analysis

Culicoides species abundance

Samples were screened using a stereomicroscope (Leica 2000) at 10.5 fold magnification in order to count and identify *Culicoides* species using wing morphology as described by Eric Denison (personal communication, Institute of Animal Health, Pirbright). Some males, of which only few were collected, were identified to species level (*Culicoides scoticus*, *Culicoides obsoletus* and *C. pulicaris*), and females were classified into three groups: 1) *Obsoletus* group (subgenus *Avaritia*), 2)*Pulicaris* group and *Punctatus* group (subgenus *Culicoides*) and 3)'other *Culicoides*' species group (including *Grisescens* group (subgenus *Culicoides*), subgenus *Beltranmyia*, subgenus *Monoculicoides*, subgenus *Silvaticulicoides*, *Festivipennis* group, *Cataneii* group, *Furcillatus* group, *Kibunensis* group and finally an *Odiatus* group). For most collections, all specimens collected were counted, however, when the *Culicoides* population was estimated to be more than 1000 *Culicoides*, the sample was subdivided into aliquots of which only one was counted and the final number of specimens was extrapolated.

Culicoides species identification

For the taxonomically identification using the determination key of Delécolle (Delécolle, 1985), the specimens were fixated and slide mounted using the method described by Wirth and Marston (1968).

PCR analysis

Samples were analysed by PCR with a set of primers (Cul-Imicola 5'-ATTACAGTGGCTTCGGCAAG-3' and PanCulR 5'-TGCGGTCTTCATCGACCCAT-3') being specific for the species *C. imicola* using method described (Cetre-Sossah et al., 2004).

Results

Culicoides species abundance

A total of 63 and 46 samples for 2005 and 2006, respectively, were analysed. For both years, samples were composed of 96% and 98% of the *Culicoides obsoletus* group, respectively, whilst the remainder species mainly belonged to the *Pulicaris* and *Punctatus* groups (Fig. 7.2).

Collections made in October showed a decrease in *Culicoides* numbers which could be correlated with the mean temperature at all the trapping sites (data not shown). No *C. imicola* species were collected at any of the sites. For the seven sites in Ticino, 27,256 *Culicoides* species were counted for 2005, whilst in 2006, a total of 43,527 midges were captured.

Culicoides species identification

Of the 1017 specimens slide mounted 25 species of *Culicoides* were identified microscopically: *Culicoides achrayi* (Kettle & Lawson), *Culicoides cataneii* (Clastrier), *Culicides chiopterus* (Meigen), *Culicoides circumscriptus* (Kieffer), *Culicoides deltus* sensu stricto (Edwards), "*Culicoides deltus variety lupicaris"* (taxonomic status uncertain), *Culicoides dewulfi* (Goetghebuer), *Culicoides festivipennis* (Austen), *Culicoides flavipulicaris* (Dzhafarov), *Culicoides furcillatus* (Callot, Kremer & Paradis), *Culicoides grisescens pro-parte,* "*Culicoides grisescen*s atypical" (taxonomic status uncertain), "*Culicoides grisescens* intermediate form" (taxonomic status uncertain), *Culicoides indistinctus* (Khalaf), *Culicoides kibunensis* (Tokunaga), *Culicoides lupicaris* s.s. (Downes & Kettle), "*Culicoides lupicaris* variety *deltus"* (taxonomic status uncertain), *Culicoides nubeculosus* (Meigen), *C. obsoletus* (Meigen), *Culicoides pallidicornis* (Kieffer), *C. pulicaris* (Linnaeus), *Culicoides punctatus* (Meigen), *"Culicoides remmi"* (taxonomic status uncertain), *C. scoticus* (Downes & Kettle) and *Culicoides subfasciipennis* (Kieffer).

In Table 7.2, the occurrence of *Culicoides* species is shown by trap site. *Culicoides obsoletus* and *C. scoticus* were the most wide spread species (found at all sites), followed by *C. pulicaris*, *C. puctatus* and *C. furcillatus* (5 sites). Species not previously recorded in Switzerland were: *C. cataneii* (1 site), *C. flavipulicaris* (1 site), *Grisescens group* (2 sites), *C. indistinctus* (1 site) and *C. nubeculosus* (2 sites); and finally, the presence of *C. dewulfi* (3 sites), *Obsoletus* group and *C. pulicaris* was established.

PCR Analysis

All 55 samples analysed (38 and 17 samples for 2005 and 2006, respectively) by PCR were positive in the genus-specific PCR but negative in the assays using *C. imicola*-specific primers.

Discussion

Culicoides species abundance

The numbers of *Culicoides* collected were relatively higher in 2006 compared to 2005 (Fig.7.2). This is most likely attributed to the higher mean temperature in 2006 including an unusually warm September in Switzerland, as well as other parts of Europe (Swiss Meteorological Office). A comparison of values for sites as well as the mean for all meteorological stations throughout Switzerland (Ronderos *et al.*, 2003)(Swiss Meteorological Office) showed marked differences in mean minimum and maximum, respectively, between the two years, which could have increased birth rate of *Culicoides* in the warmer year. For example, in Ticino (Locarno) the average yearly temperature was 12.4°C and 13°C in 2005 and 2006 respectively, with similar patterns for the whole country (data not shown). Other factors such as wind, average temperatures and humidity levels in the winter period played an important role in the over-wintering of the vectors, which possibly affected their abundance levels the following year. Recent evidence in the northern European BT outbreak suggests an average time lag of four weeks between climatic events or changes and its effect on *Culicoides* number (Anonymous, 2007). This is consistent with our data displaying a relation between the number of insects caught in relation to the mean maximum and minimum temperature curves, as seen in the high and low temperatures recorded in July giving rise to a high number of captured insects in September 2006. The temperature differences were less extreme in 2005, possibly explaining the smoother curve in vector abundance for that year. Recent trapping results from northern Switzerland showed a similar *Culicoides* composition with a high number of *C. obsoletus* present comparable to the catches made in the canton of Ticino (data not shown).

Culicoides species identification

Little data were found describing the *Culicoides* population composition in Switzerland. Ceratopogonidae in Switzerland have received modest attention from collectors and their biology is poorly understood. Only restricted geographical areas have been intensively surveyed and with the Ceratopogonidae fauna of many regions remains largely unknown (Merz *et al.*, 2002). Habermacher (1984) listed 22 species recorded in the region of Basel (northern Switzerland) where the presence of the novel potential BTV vectors, recently described, were observed: *C.*

obsoletus, *C. scoticus*, *C. dewulfi* and *C. pulicaris.* The Diptera checklist of the *Fauna Helvetica*, based on a study of sparse material, listed 17 (2002 last updating) comprising the *Obsoletus* group (Merz *et al*., 2002). Another source of information is a vector survey conducted in the Ticino region as well as in western and eastern parts of Switzerland during 2003 (Cagienard *et al.*, 2006), although the analysis was based mainly on wing morphology which is limited in providing detailed identifications.

In the present study 25 species were identified microscopically, mostly already described in the work conducted by Cagienard *et al*. (2006). During the current study difficulties were encountered in the identification of some specimens belonging to the *Grisescens* group (Subgenus *Culicoides*). Two groups of specimens, termed "*C. grisescens* intermediate form" and "*C. grisescens* atypical" were different from the *C. grisescens's* typical form included in the collection from the natural history Museum of Strasbourg (MZS). The questions which needed to be addressed concerned the presence of variations within the *C. grisescens* species or the presence of an undescribed species. A final decision was not possible based on the morphology of the adult female alone, without seeing the adult males, which unfortunately were not captured. The "*C. remmi"* from Ticino (adult female) was similar but not identical to *C. remmi* (adult male) described by Damian-Georgescu (Damian-Georgescu, 1972). In addition, *C. remmi* is considered as synonymous to *C. grisescens* (De Liberato *et al.*, 2003) (www.faunaeur.org), but in our observations, the adult female palps were very different between the two specimens classified as *C. remmi* from Ticino and *C. grisesce*ns specimens from France. Hence, whether or not the *C. remmi* from Ticino is a previously undescribed species cannot be assessed at this point in time because a decision without seeing the adult males of this species is not recommended (Dallas *et al.*, 2003). A similar problem occurred with the specimens identified as "*C. lupicaris* variety *deltus"* and "*C. deltus* variety *lupicaris"*. All these specimens should be analysed molecularly to establish their correct position in terms of phylogeny. However, this study has confirmed the absence of *C. imicola* yet has shown that the alternative potential BTV vectors are highly abundant, specifically *Obsoletus* group (*C. obsoletus, C. scoticus* and *C. dewulfi*) and *C. pulicaris*.

In Table 7.2, we observe that the different *Culicoides* species detected were distributed throughout the Canton Ticino, in terms of the southern (sites 1, 2, 3, 5 and 7) and northern parts (4 and 6) of the Canton. Site 4 represented the farm displaying the largest diversity of *Culicoides* species. The differences in population composition and distribution could be explained by the

varying altitudes, host type present and minor differences in average temperatures and habitat, which would be interesting to analyze further.

In conclusion, this study has expanded the current knowledge of the Swiss *Culicoides* population composition in the southern part of the Switzerland and has brought to light the presence of certain species recorded for the first time in Switzerland: i.e. *C. cataneii*, *C. flavipulicaris*, *C. indistinctus*, *C. nubeculosus* and *Grisescens* complex. It is important to note that *C. flavipulicaris* is a species originating from Azerbaijan which underlines the real risk of establishment of other exotic species and their potential of spreading new diseases. Since *C. imicola* is not present in Switzerland, it is important to monitor other potential competent BT vectors in an attempt to limit the potential incursion and spread of the disease, as well as expanding the epidemiological knowledge of BT in Europe.

Acknowledgments

The authors would like to thank the farmers involved for their cooperation, Michele Abderhalden, Lucia Pollina and Filippo Rampazzi (Natural History Museum, Lugano), and Jeannine Hauri from the Institute of Parasitology.

Table 7.1. Characteristics of the 7 farms included in the entomological surveillance for *Culicoides* spp. in Southern Switzerland from 2005-2006.

Farm ID	**Location**	**Grid reference Latitude (Lat) Longitude (Long)**	**Altitude (m)**	**Farm animals**
1	Muzzano	Lat -46.005377° Long -8.911665°	286	30 bovines
2	Serpiano	Lat -45.9099° Long -8.926123°	598	15 bovines
3	Novazzano	Lat -45.832468° Long -9.005224°	262	40 bovines
4	Moleno	Lat -46.269837° Long -8.993009°	253	84 bovines
5	Genestrerio	Lat -45.847096° Long -8.959502°	359	29 equines
6	Sementina	Lat -46.179716° Long -8.993974	216	24 equines
7	Meride	Lat -45.895281° Long -8.94499°	601	17 bovines 50 caprines

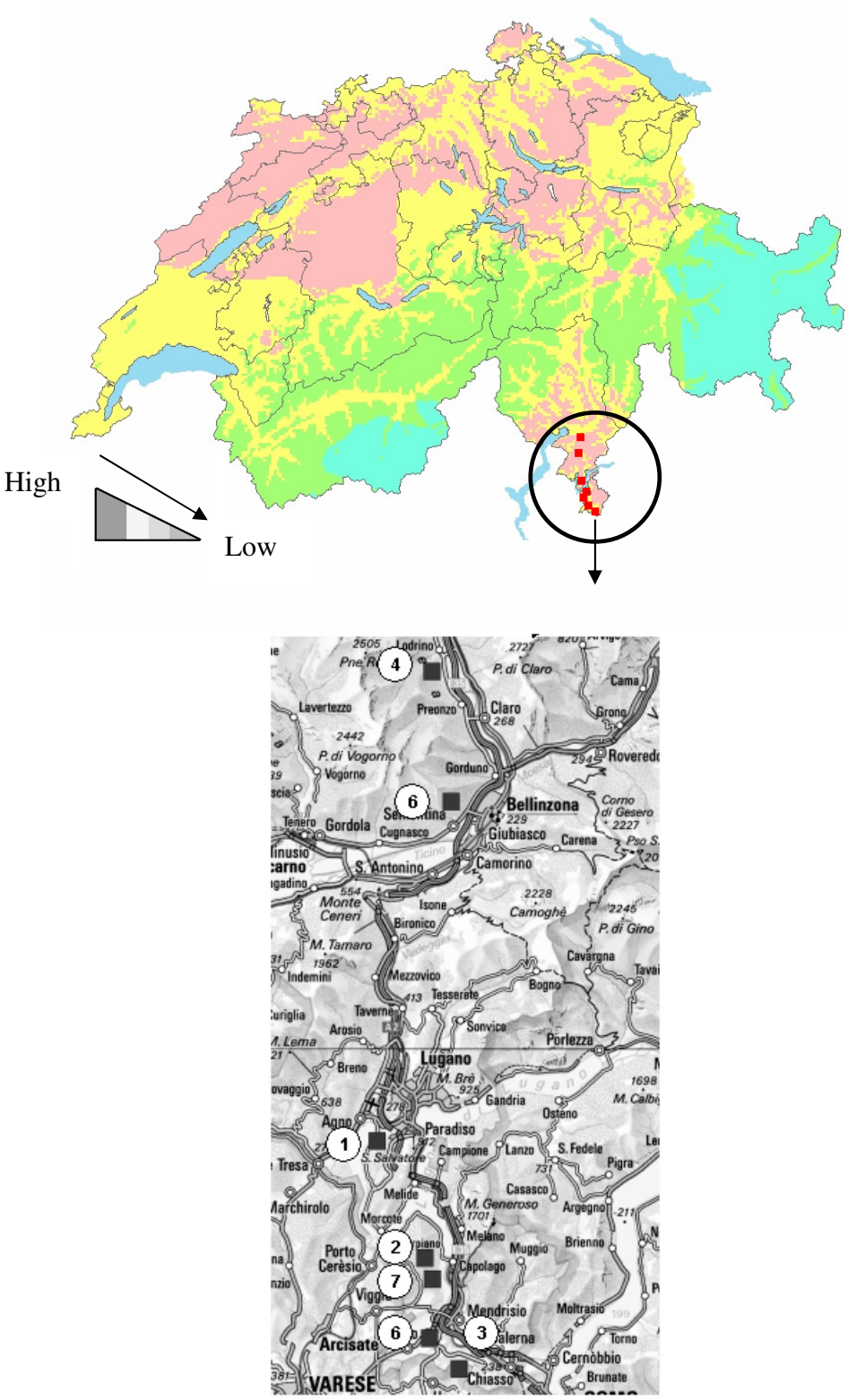
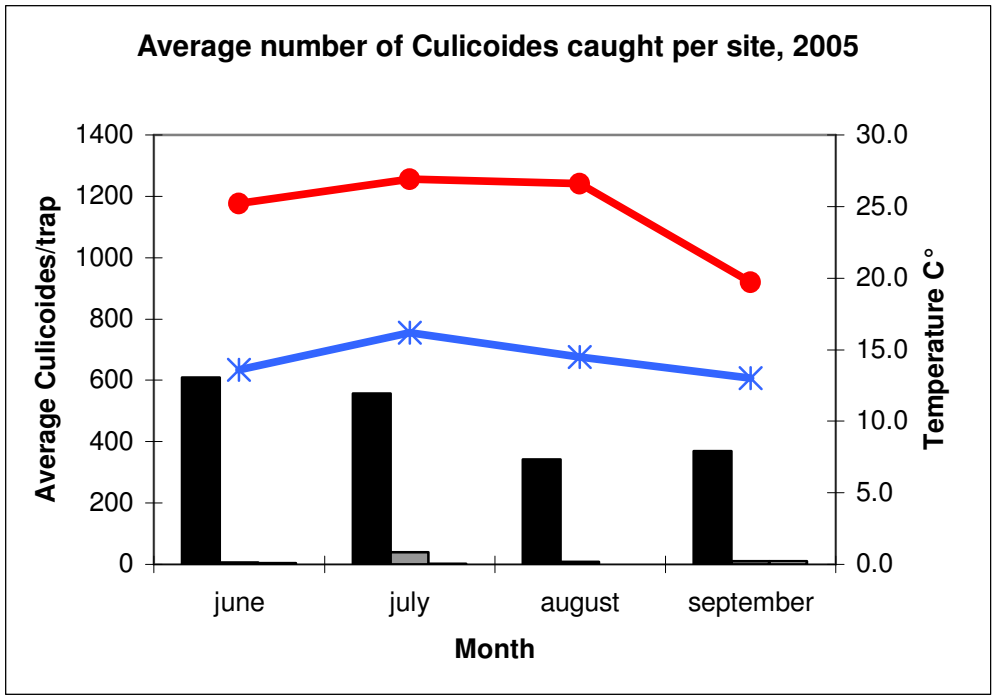


Figure 7.1: Location of trapping sites in canton Ticino. Boxes indicate location of sentinel farms. Number of site corresponding to Table 1. Map of Switzerland represents generalized vector suitability areas based on temperature, altitude and humidity values for 2006 (Racloz *et al*., 2007).

a)



b)

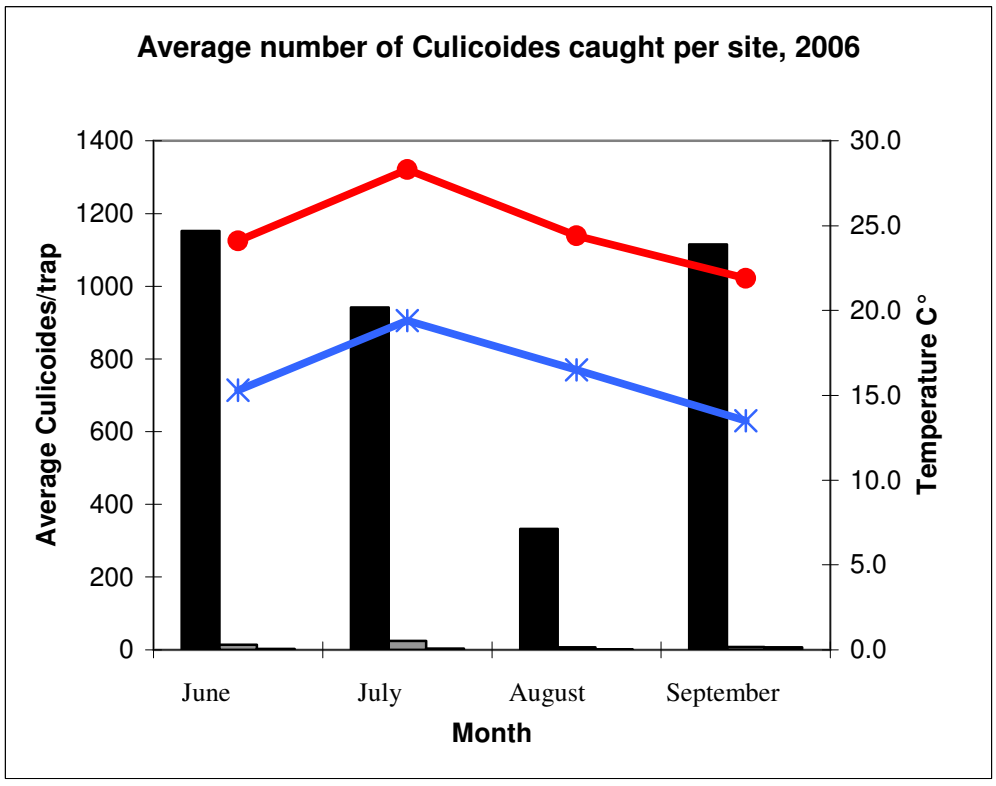


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Figure 7.2: Mean number of *Culicoides* caught per trapping site for a) 2005 and b) 2006 in relation to temperature maximum average T° minimum average T°

Table 7.2. Occurrence of *Culicoides* spp. collected in 7 trapping sites in southern part of Switzerland (Ticino) in 2005 and 2006

Species **Trapping site ID**	**1**	**2**	**3**	**4**	**5**	**6**	**7**
C. achrayi				+			
*C. cataneii**			+				
C. chiopterus			+		+		
C. circumscriptus			+				+
C. deltus		+		+			+
*C. deltus var lupicaris****		+		+		+	+
C. dewulfi	+	+					+
C. festivipennis			+	+			
*C. flavipulicaris**						+	
C. furcillatus	+	+	+			+	+
*C. grisescens atypical***				+		+	
*C. grisescens intermediate form***				+			
*C. grisescens pro-parte**				+			
*C. indistinctus**			+				
C. kibunensis			+				
C. lupicaris				+			+
*C. lupicaris var deltus****							+
*C. nubeculosus**	+			+			
C. obsoletus	+	+	+	+	+	+	+
C. pallidicornis	+	+	+	+			
C. pulicaris	+	+	+	+			+
C. punctatus	+	+		+		+	+
*C. remmi****				+		+	
C. scoticus	+	+	+	+	+	+	+
C. subfasciipennis			+				

+ represents presence on site***new species** detected in Switzerland; **taxonomic status uncertain (*C. grisescens* or new species; see discussion); ***taxonomic status uncertain (*C. grisescens*, *C. remmi* or new species; see discussion);**** taxonomic status uncertain

CHAPTER 8

Estimating the temporal and spatial risk of bluetongue related to the incursion of infected vectors into Switzerland

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This paper has been submitted to BMC Veterinary Research

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Abstract

The design of veterinary and public health surveillance systems has been improved by the ability to combine Geographical Information Systems (GIS), mathematical models and up to date epidemiological knowledge. In Switzerland, an early warning system was developed for detecting the incursion of the bluetongue disease (BT) virus and to monitor the frequency of its vectors. Based on data generated by this system, GIS and transmission models were used in order to determine suitable seasonal vector habitat locations and risk periods for a larger and more targeted surveillance program.

Thematic maps of combined temperature, humidity and altitude were created to visualize the impact which climate and the environment had on *Culicoides* vector habitat locations. Combined with estimated basic reproduction number (R_0) transmission rates, the results supported evidence that outbreaks involving vector-borne diseases were highly dependent on a variety factors with host and vector presence along with climate and altitude being of most importance. The maps highlighted the northern part of Switzerland as highly suitable for both vector presence and vector activity rates.

Results show that R_0 values were highest between June and July of 2006 as compared with the year 2005, and suggested that Switzerland was not protected from an eventual outbreak of BT, especially if the incursion arrived during a suitable vector activity period, which proved to be the case with the recent outbreaks of BT in northern Switzerland.

The results stress the importance of environmental factors and their effect on the dynamics of a vector-borne disease. In this case, results of this model were used as input parameters in creating a national targeted surveillance program which could be tailored to both the spatial and the temporal aspect of the disease and its vectors. In this manner, financial and logistic resources can be used in an optimal way through seasonally and geographically adjusted surveillance efforts. This model can therefore serve as a tool concerning vector-borne disease of veterinary relevance, as well as forming the model basis of important human zoonotic vectors which are likely to come into Europe, and in particular Switzerland.

Keywords: Maps, basic reproduction number, bluetongue, Switzerland

Introduction

Bluetongue disease virus (BT) is a vector-borne, infectious but non-contagious animal pathogen. This emerging disease affects all ruminants and has been responsible for an unprecedented continuous European epidemic which has been occurring for the past decade (Purse *et al.*, 2005). Belonging to the Orbivirus genus and Reoviridae family, there are currently 24 recognized serotypes transmitted globally by a multitude of Culicoides midge species, each with their own habitat preferences and geographical distribution albeit with the occurrence of some overlapping . Several serotypes, mostly affecting sheep, have been circulating in the Balkan and Mediterranean areas since the late 90's, which could be predicted by the advance of its Old World vector *C. imicola*. Yet an outbreak of BT serotype 8 (BTV-8) in 2006, which was last recorded in the African and the Caribbean region (Mo *et al.*, 1994), suddenly occurred in Northern Europe, an area previously free of this disease (Mehlhorn *et al.*, 2007). Preceding this event, outbreaks had been reported on a regular seasonal basis in southern Europe, mainly the Mediterranean region involving several serotypes namely BTV -1, -2, -4, -6, -9 and -16 (Purse *et al.*, 2005).

At the time of writing, BTV-8 was reported in northern Switzerland in the canton of Basel-Stadt. A few days later a further farm was affected in the canton of Solothurn, followed by a case in Basel-Land. To date, a total of 12 cattle and two goats have tested positive for BTV-8 on seven different farms. Although no firm conclusion have yet arisen as to the cause of these cases, wind direction patterns along with temperature records of these areas suggest possible intrusion of infected vectors originating from the surrounding BT affected areas.

Due to the dynamics of the pathogen, combined with the fact that the geographical limits of other vector-borne diseases are also expanding, Switzerland conducted a nation-wide survey in 2003 to determine the status of BT disease and the presence of its vectors (Cagienard *et al.*, 2006a). Although this resulted in proving freedom of disease, the presence of vector species competent of transmitting BT were found to be abundant in certain areas of the country. This in turn prompted the establishment of a sentinel herd surveillance system through serological and entomological monitoring, focusing on certain areas of the country (Purse *et al.*, 2004b, Racloz *et al.*, 2006b). Due to the nature of the vector borne diseases, and the fact that the BT was not yet present in Switzerland, an early warnings system was created through risk based sampling (Racloz *et al.*, 2006b). This involved identifying geographical areas which match habitat criteria

for the eventual survival and establishment of a vector species, which is often also linked to host presence (Racloz *et al.*, 2006a), and was determined through the production of GIS maps (Racloz *et al.*, 2007). However these maps were limited to yearly observations, and could not predict at what stage the highest peak of vector activity would occur on a temporal basis, nor differentiate between rainfall and snow data in certain months. Hence a more informative and detailed mapping method alongside a mathematical model was created. Similar objectives also using maps as predictive tools have been used in other countries such as Italy, Spain, and France concerning BT disease and its vectors (Purse *et al.*, 2004b, Conte *et al.*, 2005, Guis, 2007).

The aim of this study was to combine GIS maps with data collected in the field and integrated in a mathematical model to explore the spatial and temporal areas more prone to 1) the establishment of important vector populations, and 2) enabling the spread of the disease due to the nature of geographical and climatic features. The basic reproduction number is described as the 'expected number of secondary cases that would arise from a typical primary case in a susceptible population' (Roberts *et al.,* 2003), as has been calculated for other vector-borne diseases such as Malaria (Smith *et al.*, 2007), West Nile (Wonham *et al*., 2004), African Horse disease (Lord *et al.*, 1996), as well as recently for BT (Gubbins *et al.*, 2007).

Methods

GIS mapping

Separate thematic maps were created using ArcGis (Version 8.3 , Environmental Systems Research Institute, Inc.) for monthly average temperature, altitude and humidity for the years of 2005 and 2006 using data from 50 meteorological measuring stations provided by the Swiss Meteorological Office, as previously described (Racloz *et al.*, 2007). The aim was to create combined monthly vector suitability maps using these parameters to visualize the variation in potential risk areas during each season. Once monthly datasets were incorporated into the map, smoothing out was performed through kriging, apart from the altitude map which was derived from an elevation model. Suitability categories, based on *Culicoides obsoletus* group biology and habitat data (Mellor *et al.*, 2000, De Liberato *et al.*, 2005, Purse *et al.*, 2005, Carpenter *et al.*, 2006, Osmani *et al.*, 2006, Purse *et al.*, 2006), were used to reclassify the output values, in order to grade all monthly maps on a standard scale. The 'environmental envelope' of the *Obsoletus*

group of *Culicoides* was concentrated upon in contrast to the classical Old World vector *C. imicola*, due the fact that the former is the most abundant group caught in the Swiss entomology surveillance program (Racloz *et al.*, 2006b) and has been shown to transit BT virus in other countries (Mehlhorn *et al.*, 2007). The maps were then layered together using the addition function in the raster map calculator which created a single combined suitability map for each month. Whereas previous maps for Switzerland had not distinguished between rainfall and snow in the precipitation category, the production of more detailed and individual maps allowed incorporating this difference into the analysis.

R_0 calculations

The second part of the study was to determine the potential consequence of a bluetongue outbreak, using the basic reproductive number (R_0) and incorporating local climate data as well as Swiss *Culicoides* abundance information. The latter information deriving from entomological data collected using Onderstepoort blacklight traps in sampling sites for the years 2005 and 2006. Minimum and maximum temperature during trapping, insect abundance and diversity, host species present and altitude for each trap location were also recorded and monthly averages for temperature were obtained from the Swiss Meteorological Office. Based on previous Malaria (Smith *et al.*, 2007a) and West Nile models (Wonham *et al.*, 2004), as well as a recent publication on BT R_0 (Gubbins *et al.*, 2007), hypothetical transmission values representing new BT cases per month for both years were estimated using the following equation. Values and symbols used in the equation are explained in Table 8. 1.

$$R_0 = \frac{mab_1}{r+\lambda} * e^{-\mu\tau} * \frac{ab_2}{\mu}$$

In terms of vector-borne diseases, the basic reproduction number (R_0), is defined as the number of new infections that would result from the introduction of a single infectious vector specimen into a completely susceptible/naive population of hosts (Wonham *et al.*, 2004). R_0 values were estimated for each month for the years 2005 and 2006 incorporating the following factors: vector density (m), derived from *Culicoides* catches in the national entomological surveillance, as well as values obtained from literature and current field data regarding the extrinsic incubation period (τ), vector biting rates (a), vector death rates (μ), host death (λ) and

recovery rates (r), transmission rates from vector to cattle (b_1) and vice versa (b_2) (see also Table 8.1). The R_0 values were plotted against monthly minimum, average and maximum temperatures for the area where entomological trapping occurred (Fig. 8.1a and b).

In order to visualize the results in a similar method as described above for the suitability maps, further 'consequence' maps were created using the R_0 values to produce monthly maps for 2006. The R_0 values were divided into four categories as previously mentioned, and reclassified to share a standardized scale for each month, as well as being comparable to the suitability map scales. The two sets of maps were then combined by adding the respective layers for each month through the raster map function to produce final combination maps incorporating both spatial and temporal factors (Fig.8. 2).

Results

GIS mapping

The combined result of the suitability maps and the R_0 maps for the months of January, July, September and October for 2006 is shown in Figure 8.2. The suitability maps (Fig 8.2, left) highlighted the localized microclimates occurring in Switzerland as well as the importance of the role which the alpine area plays in separating the northern and southern parts of the country as seen in the different risk categories for each month. The maps showed that some areas remain slightly suitable for Culicoides survival (suitability maps) in the colder seasons and the prime conditions for Culicoides activities were seen in the month of July. In comparison, the R_0 maps showed negligible risk of BT spread in the colder months of 2006 (Fig.8.2, middle). The difference between the vector suitability maps and the calculated transmission values are especially when comparing the maps for July and September. July seems to be a less suitable month for vectors yet R_0 values are higher than in the month of September, where more areas are considered high risk zones for Culicoides activity. However when combined, the risk maps (Fig. 8.2, right) showed that the risk of an outbreak or spread of BT in Switzerland was not negligible even in the colder seasons.

R_0 calculations

The monthly R_0 values along with temperature records between the years 2005 and 2006 were considerably different. Figure 8.1a shows that the R_0 peak for 2005 occurred during June

and July with a maximum value of 19, as compared to 2006 (Fig 8.1b) which had two R_0 peaks occurring in late May and August reaching R_0 values of 22 and 16 respectively. For 2005, June proved to be the warmest month, with an average temperature of 20.7°C as compared to 2006 (Fig 8.1b), where the warmest month of July recorded an average monthly temperature of 25.7°C. Similarly, the month of February in 2005 recorded the lowest average minimum temperature of –7.6°C whilst in 2006 a minimum of –6.5C° was recorded in January. The two relatively large differences seen between the years were 1) a much warmer winter period of 2006 as well as 2) higher temperatures during the summer of the same year. Monthly variations were also seen in both years following seasonal patterns as shown in the suitability maps in Figure 8.2 (data only for 2006).

Discussion

Our results showed that the months of July and September 2006 were the most suitable period in Switzerland for vector activity in terms of climatic conditions (suitability maps), while the highest R_0 value occurred in June. This is an interesting finding in relation to the BT incursion and subsequent outbreak in Northern Europe, which began in 2006, possibly indicating similar conditions in North Europe. A recent study by (Gubbins *et al.*, 2007) has also assessed the risk of BT in the United Kingdom using the basic reproduction number. Similarly to their findings, the R_0 values were highest when temperatures were between 15°C-25°C. In our study though, only cattle farms and the effect of BTV-8 were assessed due to relatively larger density of cattle in Switzerland. Due to its climatically diverse zones, a similar model could benefit the southern part of Switzerland although sheep, as well as focusing on other BTV serotypes common in Italy. The ultimate aim of determining R_0 values was to use the outcomes as a source of input parameters in creating a national targeted surveillance system to detect the primary occurrence of BT into Switzerland

The basic reproduction number (R_0) for vector-borne diseases is a more complex number to calculate due to the influence of seasonal fluctuations (Bacaër, 2007), local climate and environmental features as well as the abundance of breeding sites available near hosts which affect vector dynamics (Lord *et al.*, 1996). The transmission rates of the disease will also change depending on temperature factors affecting vector to host or host to vector interaction, along with the extrinsic incubation time, biting rates and vector mortality rates (Gubbins *et al.*, 2007). In this

study, vector density numbers along with temperature values were used from field data collected throughout the project in order to produce R_0 values specific to the areas and time frame studied.

For Switzerland, the peak of R_0 can be explained by the fact that the largest amount of *Culicoides* midges were recorded in June, probably due to optimal breeding and hatching conditions as May was a mild and humid month. July held the record for maximum temperature, which leads to higher vector activity and successful virus transmission. However high temperatures also increase vector mortality rate and thereby lower R_0 values for this month. Due to the very different meteorological patterns in the past three years, we would therefore expect that maps and R_0 for 2007 to be quite different compared to 2006.

Due to the lower temperatures recorded in August 2006 and a hot July, transmission values as well as vector density decreased significantly. In terms of the Northern European BTV8 outbreak data, this would match reports stating that the maximum number of cases occurred in October which would originate from a high number of vectors present a few weeks previously. Due to the nature of *Culicoides* development, it has been suggested that cases occur at a time lag of circa four weeks from peak vector density periods, which corroborate the evidence from the trapping data and the transmission values (Anonymous, 2007). It can also be assumed that although the first cases of BT were detected in August in The Netherlands, due to the high maximum temperature recorded in July, the number of vectors along with their competency and capacity could have been primed for an effective transmission period, as observed in many other affected countries (Anonymous, 2007). Comparing the two years 2005 and 2006 in Switzerland for wind speed, rainfall and relative humidity (data not shown) did not reveal differences as clear as those for temperature with the exception of a higher level of rainfall recorded in March 2006 which could have provided more humid areas appropriate for breeding grounds.

A limiting factor in our model is that vector density data resulted from actual trapping sites, and since the program was created as an early warning system, only high risk areas were targeted for entomological surveillance. In these areas high numbers of vectors were expected. Therefore the transmission values may be overestimated in some parts of the country. Another factor to consider is, as mentioned previously, only the dynamics between BTV-8 and the vectors belonging to the *Culicoides obsoletus* group were studied. Outbreaks involving other BT serotypes and their dynamics in *Culicoides* vectors produce different R_0 values (Venter *et al.*, 1998). They may also have a different preference for distinct geographic and climatic conditions. Such differences have been described for the behavior of BTV-2 and *Culicoides imicola* in

southern France (Breard *et al.*, 2004), and the role of *C. imicola* in South Africa (Venter *et al.*, 1996).

The recent cases of BT in Switzerland in the month of October 2007 occurred in the northern part of the country. Temperatures for the affected region were similar to those of 2006, and the number of cattle affected on the three farms correspond to the R_0 figures calculated for that area and time period. Out of a total of 608 susceptible cattle from the affected farms, it was detected in 12 animals, with prevalence rates ranging from 0.63% to 10.3%.

The use of GIS in spatial analysis and mathematical modeling have been increasing in the last decade both in prospective, as in this case, and retrospective manners as seen in similar approaches concerning BT outbreaks in Italy and Spain (Purse *et al.*, 2004b, Pili *et al.*, 2006). Our findings highlight the potential for establishing a flexible surveillance system taking into account environmental factors. In a targeted surveillance system, this could mean increased serological testing during a specific warmer period or in specific geographical areas. Given the lack of epidemiological data available for Switzerland concerning BT cases, the creation of thematic and risk maps on a monthly and annual basis, illustrated the variability in the behavior of vector borne diseases and the possible consequences of virus introduction. It also provided the basis for creating a surveillance system that is targeted at high-risk regions and months. In the case of Switzerland, the maps and R_0 were used as input parameters for the creation of a BT surveillance scenario tree (Hadorn & Stärk submitted,(Martin *et al.*, 2007), with aim of comparing the effectiveness of alternative surveillance system designs. A risk-based surveillance program was implemented in July 2007 consisting of three surveillance system components ; the serological bulk milk testing of 200 sentinel herds located in areas considered of higher risk to BT occurrence, as well as clinical surveillance programs for cattle and sheep farmers throughout the country.

Conclusions

GIS mapping techniques along with support via statistical and mathematical models can help improve disease surveillance and control methods by providing a basis for targeting the efforts. This includes the ability to improve prediction maps once more comprehensive field data has been collected, and adjust surveillance efforts in a timely and accurate manner. Flexible

surveillance program should be used in order to attribute financial and human resources to high-risk areas considering temporal and spatial factors.

Acknowledgments

VR would like to acknowledge Dr. Patrick Presi and Dr. Heinzpeter Schwermer for their help in GIS matters, as well as Ms Monika Kuhn and Dr. Simona Casati for their contribution to the entomological field work.

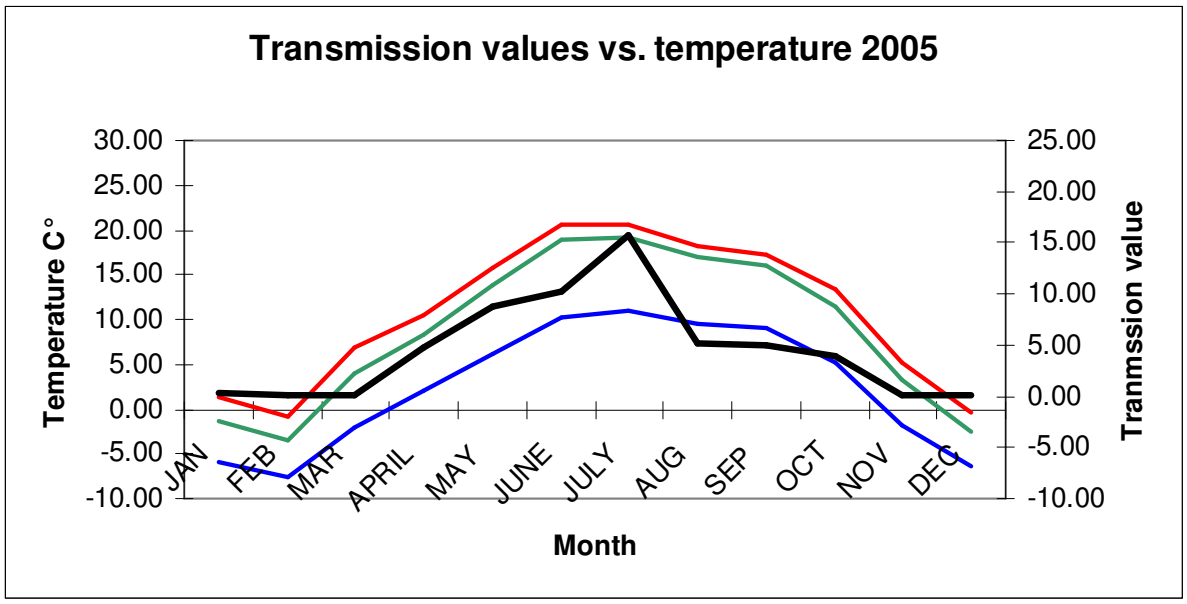
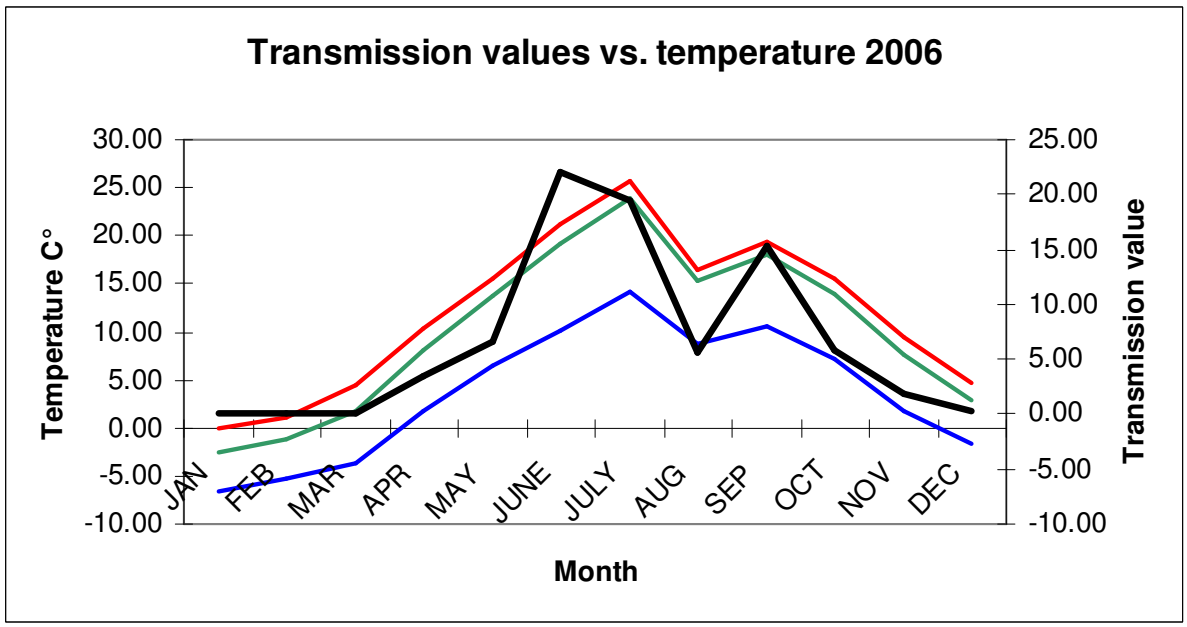


Figure 8.1: a) Calculated R_0 values for 2005 in Switzerland (secondary y-axis).along with monthly average, minimum and maximum temperatures (primary y-axis)



b) Calculated R_0 values for 2006 in Switzerland (secondary y-axis).along with monthly average, minimum and maximum temperatures (primary y-axis)

Table 8.1: Symbols and their biological meaning used to calculate BT transmission values for Switzerland

Symbol	Unit	Biological meaning	Values	Reference
m	midge/trapping night	Vector density	range	(Racloz et al., submitted)
a	bite /day	Vector biting rate	0.25	(Gubbins *et al.*, 2007)
b_1	successful bites/midge	Transmission from cattle to vector	0.01	(Mellor *et al.*, 2000)
b_2	%infectious bite /infected host	Transmission from vector to cattle	0.9	(Carpenter *et al.*, 2006)
r	cattle/ day	Recovery rate of cattle	0.04	(Anonymous, 2007)
λ	cattle/ day	Cattle death rate	0.00008	(Anonymous, 2007)
e		2.718	2.718	Universal value
μ	vector/ day	Vector death rate	range	(Baylis *et al.*, 1998)
r	days	Extrinsic incubation period	range	(Mellor *et al.*, 2000)

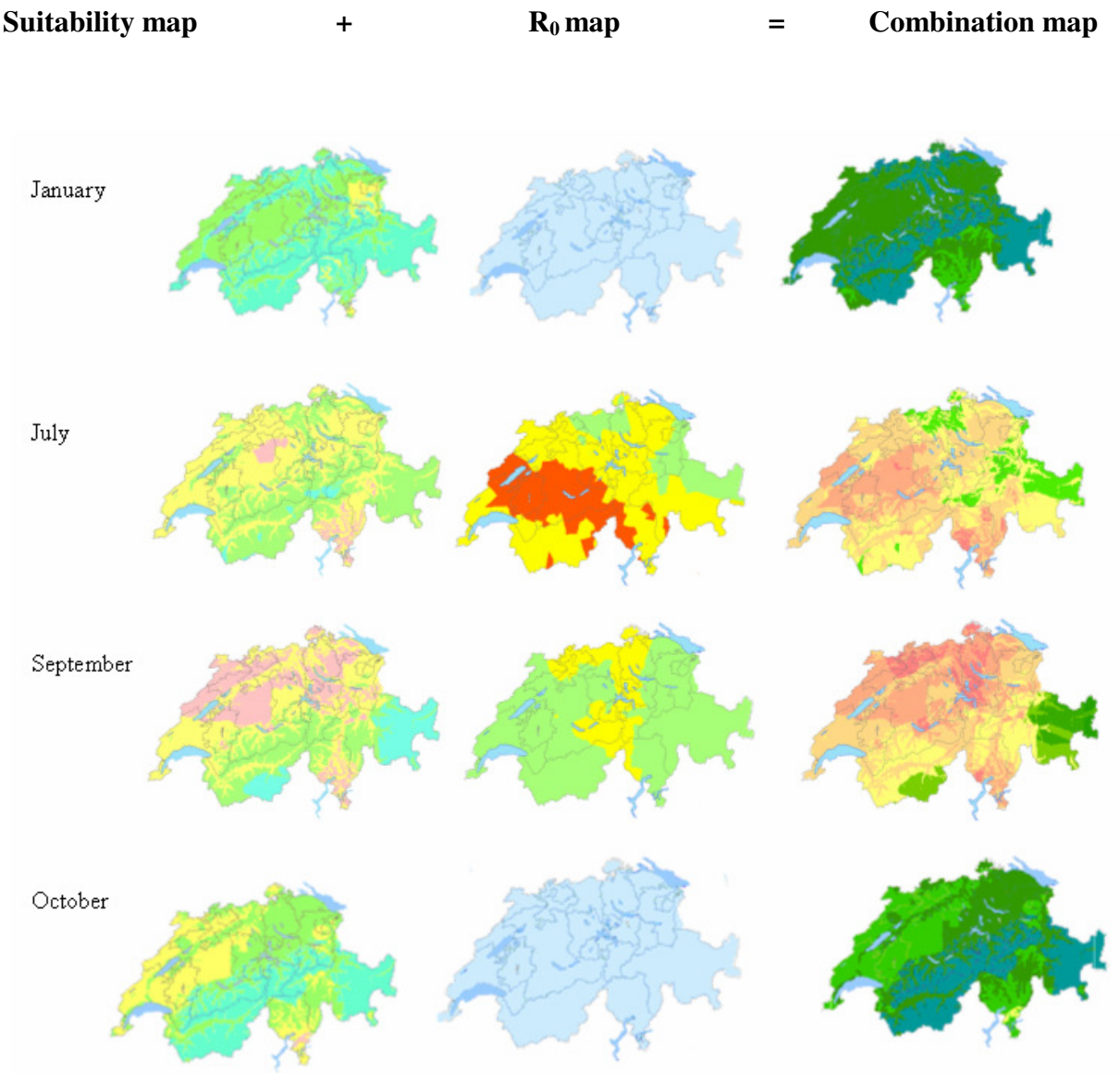


Fig. 8.2 : Suitability maps (left margin) which were added to R0 maps (middle margin) to create final combinations maps (right margin). Selected months of January, July, September and October are shown.

CHAPTER 9

Establishing a national surveillance system for bluetongue in Switzerland using Scenario Tree modelling

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Work in progress

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SUMMARY

Although Bluetongue disease has a world wide distribution, Northern Europe detected its first cases in 2006. Previous to these outbreaks, BT was restricted to the Balkan and Mediterranean areas where it was present on a regular basis over the past decade . Bluetongue disease is an economically important disease which affects domestic and wild ruminants. Belonging to the Reoviridae family, there are currently 24 known serotypes of this arbovirus, each with varying mortality rates as well as vectors; species belonging to the *Culicoides* genus.

Due to the emerging presence of bluetongue disease in countries neighbouring Switzerland, a national survey was carried out in 2003 in order to determine the BT status in cattle, as well as to examine the abundance and frequency of the vector species throughout the country . Results from this study prompted the establishment of a sentinel herd surveillance program with aim of serving as an early warning system for the primary incursion of BT disease through infected *Culicoides* insects . This sentinel surveillance program set up between 2004-2007, and due to the detection of BT in Northern Europe in 2006, a revision of the surveillance strategy was called for, taking into account the BTV-free status of Switzerland.

Combining entomological, climate-based, geographic and host presence data, it was possible to identify areas which presented a potentially higher risk for the primary incursion of BT disease, as well as its spread once established . This information was used as input parameters in the creation of a Scenario Tree Model with purpose of determining a suitable combination of surveillance system components which maintained a risk-based element yet remained financially viable considering that the country was still free from BT disease.

Hence, a scenario tree model was constructed which recommended the choice of a combined sentinel bulk milk testing strategy along with a passive clinical surveillance, through the simulation of various surveillance options and their economic outcomes. These were initiated in July 2007 through the monthly testing of 200 cattle herds distributed in areas considered at higher risk, as well as efforts to increase disease awareness via the release of documentaries and BT workshops aimed at stakeholders and farmers.

Key words : Scenario Tree Model, bluetongue, Switzerland, surveillance program

INTRODUCTION

Bluetongue (BT) disease is a vector borne viral disease which until recently was restricted to tropical and subtropical areas of the world . Since 1998 with its introduction into Greece, it has also been causing outbreaks in Europe with the most recent epidemic in 2006 affecting Germany, the Netherlands, France, Luxembourg, Belgium and as of September and October 2007 spread into the United Kingdom, Denmark and Switzerland respectively. Due to the advancement of BT in Italy, and the widening distribution of the main Old World BT vector, *C. imicola*, in countries adjacent to Switzerland, an initial baseline survey was conducted as a nationwide cross-sectional study in 2003 to determine the status of BT in the country, which was confirmed as absent through the serological screening of over 2000 cattle (Cagienard et al., 2006b). In combination with the serological testing, an entomological study was performed with focus on southern, western and eastern parts of the country and resulted in the identification of potential BT vectors in many of these areas . These results in turn prompted the decision to establish an early warning system for bluetongue in Switzerland through the serological and entomological monitoring of sentinel cattle herds located in areas considered of heightened risk for both the incursion of BT vectors into Switzerland, and potential virus circulation (Racloz et al., 2006b).

An important aspect of veterinary disease surveillance is the collection and analysis of relevant epidemiological information, with aim of improving animal health by providing up to date information to decision makers and stakeholders. Due to the diversity of diseases circulating in both human and veterinary fields, it is important that a surveillance system detects or monitors the events in both diagnostically sensitive and financially viable terms. A surveillance system is a method which generates information on the health and disease status of animal populations including one or more component activities (OIE Terrestrial Animal Health Code 2005) (www.oie.int). The ability of these component activities to collect up to date epidemiological data will affect the performance of a surveillance system designed to monitor disease. Challenges for creating a risk based surveillance strategy concerning vector-borne diseases exist due to the number of factors involved: the biology of the vector, the susceptibility of the host and the environmental conditions which affect the success of disease transmission. Certain aspects of these parameters were taken into consideration within the scope of this project, keeping in mind that there are still many gaps in the knowledge of BT epidemiology.

Based on the accumulated understanding of the risk factors for BT in Switzerland and the changed BT situation in neighboring countries, the surveillance objectives for Switzerland needed to be adjusted and consequentially, the design moved from a baseline random survey to a sentinel surveillance network and finally to nationwide risk-based sampling. The objective was to design an optimal surveillance system which maintained a risk-based element and covered the whole country yet remained in a defined financial boundary, considering that the country was still free from BT. To reach this goal, a stochastic scenario tree modelling approach was used (Hadorn & Stärk, submitted, (Martin et al., 2007)) in order to determine what type of surveillance system components (SSC) were best suited in Switzerland for a national surveillance system for bluetongue in terms of performance sensitivity and financial grounds.

In this paper, we illustrate the scenario tree model for Bluetongue virus serotype 8 (BTV-8) and how local vector and host population data, along with climatic and geographical information were collected for incorporation into the model in order to make it specific for Switzerland.

During the time of writing, Switzerland experienced seven outbreaks of BT. These were located in areas considered at high risk of BT occurrence based on vector biology. In the first three farms, along with the fifth and seventh case, the identification of BT was made through a clinical suspicions followed by serological testing. The remaining two outbreaks were detected through the mentioned Bulk Milk testing, affecting single cows in the canton of Basel-Land and Vaud.

MATERIALS AND METHODS

The scenario tree methodology allows to visualise the outcome of a variety of interventions, in this case surveillance system components, by changing the input parameters (i.e. new data on a monthly basis). As described by (Martin et al., 2007), five main steps need to be followed in order to construct a scenario tree. Firstly, to determine the order of events affecting the objective of the scenario tree. Secondly to include the livestock structure of the country or area involved into the model, followed by identifying the risk factors involved in the disease/condition. The fourth step is to incorporate the testing and sampling methods used, and finally to assess the feasibility of the program (www.ausvet.com.au/freedom).

In this study, the objective of the scenario tree was to determine which surveillance system components (SSC) could be used as an effective and economical program to detect BT in Switzerland. The livestock involved in this model were cattle and sheep populations. The most complex steps were to determine risk factors involved in BT infection, along with analyzing various detection methods for each SSC as described below. Finally the input parameters were determined, and the outputs analysed on a monthly basis.

Risk factors for BT integrated in the stochastic simulation model

Bluetongue disease is a non-contagious, infectious, insect-transmitted viral disease that affects domestic and wild ruminants (Purse et al., 2005), with its occurrence exclusively related to the presence of competent vectors. Therefore, the two main risk factor for BT infection involves i) the spatial risk factors: represented as areas of the country which have suitable climatic, geographical, host associated and entomological features for the sustainement and spread of the virus. And ii) the transmission risk factors, based on local vector abundance and virus transmission rates.

i) Spatial risk factors

Climatic and geographic areas suitable for the establishment of the vector were determined through the creation of monthly thematic maps for altitude, precipitation and average temperatures using ArcGis (Version 8.3 , Environmental Systems Research Institute, Inc.), and data from 50 meteorological stations provided by the Swiss Meteorological Office for the year 2006 (Racloz et al., 2007). The separate maps were smoothed out through ordinary kriging apart from the altitude map which derived from a previous elevation model, then layered together using the raster map calculator function to form individual monthly suitability maps for 2006. Suitability categories were created through the review of literature available on *Culicoides* biology (Mellor et al., 2000, De Liberato et al., 2005, Purse et al., 2005, Carpenter et al., 2006, Osmani et al., 2006, Purse, 2006) and subsequently divided into four risk categories, ranging from high, high-medium, low-medium and low risk levels.

ii) Transmission risk based on R_0 calculations

The geographic risk areas were then used to determine the relative risk represented by each risk level in terms of vector activity and allowed to measure the scale of a potential outbreak. This was done by calculating the basic reproduction number (R_0) for each suitability risk zone. For vector-borne diseases, R_0 is considered as the number of cases a single infected vector will produce in a susceptible population of hosts (Gubbins et al., 2007) . This was used to attribute a transmission level to each geographical risk category in order to stress the efficacy of a risk based sampling. Hence, each monthly suitability map would display different areas of high to low risk zones, and a R_0 value was calculated through the input of entomological data collected through the sentinel herd surveillance program in Switzerland (2004-2007) (Racloz et al., 2006b). The R_0 equation used was based on previous malaria (Smith et al., 2007b), West Nile models (Wonham et al., 2004), as well as current knowledge of bluetongue epidemiology (Gubbins et al., 2007).

Surveillance system components (SSC)

Another important step in creating the model was to identify the different surveillance methods available for BT diagnosis, and which populations had to be surveyed. As discussed in Hadorn & Stärk (submitted) a surveillance system may be composed of both active and passive surveillance parts. The ability to combine various independent SSCs (Martin et al., 2007) and generate an overall sensitivity for the surveillance system all within an economically viable process is an important feature of scenario tree modelling, especially for emerging disease surveillance. Since the aim of this project was to establish a federal surveillance program, it was important to include criteria stated by the OIE concerning BT surveillance. Hence the model was designed to analyse the surveillance system for BT at the design prevalence of 0.2% .

The following potential SSCs were identified for BT surveillance in both cattle and sheep populations: serological random sampling of cattle and sheep, randomly selected bulk milk testing of dairy herds, abortion testing in cattle and sheep, abortion testing, isolation of virus from vectors, slaughterhouse sampling in cattle and sheep, clinical surveillance in cattle and sheep, and finally risk-based sentinel herd sampling (serological and bulk milk testing). After creating a basic scenario tree with these SSCs, the respective component sensitivities and their economic

implications were evaluated (data not shown). For economic and practical reasons, the following three SSCs were retained for further analysis: passive surveillance strategy in terms of clinical surveillance of sheep and cattle, as well as the active SSC of bulk milk testing in sentinel herds.

Clinical surveillance in cattle and sheep

As described in Hadorn & Stärk (submitted), clinical surveillance describes the detection of infected farms and animals through animal caretakers and veterinarians. The key elements in this process are the probabilities that 1) the infected animals show clinical symptoms and 2) the correct diagnostic and contact measures are taken by the farmers and veterinarians, based on disease awareness levels (DA).

Due to the nature of the northern European outbreak and the exclusive involvement of BT serotype 8 (BTV-8), it was possible to collect clinical symptoms data from affected countries and use it as an input parameter for this SSC. The disease awareness levels of farmer and veterinarian, i.e. the probabilities of the farmer contacting the veterinarian, and that of the veterinarian conducting the appropriate BT diagnostic test, which involves serological blood sampling using an ELISA , were set arbitrarily using a similar disease awareness scheme as described in Hadorn & Stärk (submitted). In the case of CLIN for cattle, it was decided to attribute a slightly higher (by 5%) DA to the farmers as compared to CLIN for sheep (Table 9.1a-b). The assumption was that cattle care is based on the individual animals compared to sheep care and therefore the probability of contacting a veterinarian is slightly higher. Similarly, at the time of writing, clinical manifestations were slightly higher in sheep than cattle; hence the likelihood that a veterinarian takes samples was set at 5% higher in the sheep population.

For the CLIN in cattle, a low DA was assigned to the months of January to June, and a low-medium DA from July to December. This was due to the increase in BT cases in Northern Europe, as well as the effect of the vector activity period. For CLIN in sheep, input parameters of DA were modified depending on the month the SCC was running under. In this case, low DA was given for the months of January to July, a low-medium DA level was given for August and September, ending with the medium-high DA levels for October to December. The differences in the DA values were chosen on the basis of media output of the Federal Veterinary Office, as well as the pattern of the vector season and finally due to the BTV-8 situation in northern Europe. Values for the different DA levels are given in Table 9.1.

In this surveillance system component, (CLIN-SSC), all geographic risk zones were taken into consideration, and the final sensitivity level for clinical surveillance in cattle and sheep was calculated considering the total number of cattle herds (37,860) and sheep (22,201) in Switzerland.

Sentinel Herd Bulk Milk testing in dairy cattle

Sentinel herd bulk milk testing means that a certain number of herds with an increased risk of getting BT-infection are tested monthly using the milk test ELISA (ID Screen® Blue Tongue Milk from ID Vet, France). In Switzerland, a regular nation wide milk sampling procedure already exists in terms of a quality testing program and the bulk milk samples for the BT surveillance program could be integrated in this procedure. Therefore, costs for bulk milk sampling proved to be much less expensive than a similar surveillance method based on serological blood sampling on the basis of single animal testing. According to the analysis of the basic model with regard to costs and system sensitivity benefit, it was decided to choose 200 herds within this program located in areas considered of higher BT and vector risk.

The input parameters used to determine the overall sensitivity Se for this SSC were 1) the risk factors, involving the distribution of the geographic risk areas as well as the relative risk for vector activity levels calculated through the basic reproduction number (R_0), and 2) the detection procedure of bulk milk testing using the commercially available ELISA. For this SSC, only the herds distributed in the high and hi-medium risk categories were considered.

Input parameters

Input parameters which needed to be calculated for every month were those of: herd distribution in the different geographic risk levels, vector activity rates to determine the relative risk of each geographic zone, disease awareness (DA) estimates, clinical symptom data and diagnostic test performances (Table 9.1c). The final process was to incorporate host distribution data and to determine the percentage of herds in each risk and suitability category on a monthly basis, also taking into consideration the altered distribution of cattle herds during summer due to the alpine pasture tradition. As mentioned before, all risk factor values were calculated on a monthly basis due to the fast moving nature of BT, as well as the effect of different climatic patterns on vector activity rates.

Values for these parameters were calculated either as fixed numbers as in the case of herd distribution and relative risk rates, or as Pert distributions for the remaining input parameters. The Pert distributions accounted for the uncertainty in the data, and permitted a range for minimum, most likely and maximum values to be calculated when running the Monte Carlo simulation.

Once all the input parameters and the risk factors had been determined for each month, separate simulations were run for each SSC by using the @Risk software program (Palisade Corporation) a Monte Carlo with 5,000 iterations. The overall combined sensitivity output was then calculated once the SSCs sensitivity values for all months were determined.

The three SSCs, namely the sentinel herd bulk milk testing and the clinical surveillance in cattle and sheep, were combined to give a final overall sensitivity value for the performance of the entire surveillance system as demonstrated in (Hadorn & Stärk submitted) (Martin et al., 2007).

Table 9.1a-c: List of input parameters and their values used in bluetongue scenario tree modelling for Switzerland.

9.1a) Disease awareness levels for clinical surveillance in cattle (CLIN cattle)

Cattle herd	**Value (Pert distribution)**		
Farmer calling veterinarian	**Minimum**	**Most likely**	**Maximum**
Low	15%	25%	35%
Medium	35%	45%	55%
Veterinarian performing test	**Minimum**	**Most likely**	**Maximum**
Low	10%	20%	30%
Medium	30%	40%	50%

9.1b) Disease awareness levels for clinical surveillance in sheep (CLIN sheep)

Sheep/mixed herd	**Value (Pert distribution)**		
Farmer calling veterinarian	**Minimum**	**Most likely**	**Maximum**
Low	10%	20%	30%
Medium	30%	40%	50%
High	50%	60%	70%
Veterinarian performing test	**Minimum**	**Most likely**	**Maximum**
Low	15%	25%	35%
Medium	35%	45%	55%
High	55%	65%	75%

9.1c) Input parameters for diagnostic procedures

Input Parameter	**Level**		
Diagnostic values	**Minimum**	**Most likely**	**Maximum**
Bulk milk test diagnostics (Se)	98%	99%	100%
Clinical symptoms (cattle)	3.9%	7%	47%
Clinical symptoms (sheep)	4.7%	15%	48%

RESULTS

Changes in the risk factors for BT were seen on a monthly basis in both the spatial risk factor and the transmission risk factor as discussed in (Racloz et al., submitted). The peak of the spatial risk occurred in the month of September, followed by August, July and May 2006 respectively. In terms of the transmission risk values, June represented the highest risk, followed by July and September. These risk factors had the largest effect on the sensitivity of Sentinel herd Bulk Milk testing SSC component, which can be seen in the fluctuations of the Se and in the R_0 values.

Diagnostic performance values for the separate SSC ranged from 0% to 80% throughout the year, but reached the highest levels in the second half of 2007. Overall, the CLIN SSC for cattle had the highest levels and a range of 35%-80%, followed by the sheep CLIN SSC which

had values from 17%- 78% whilst the sentinel bulk milk testing SSC had levels ranging from 0%-41%.

The clinical surveillance in cattle SSC had one main increase seen in late July due to the estimated increase in DA related to the release and distribution of a BT documentary to stakeholders, and the raised number of reports in Swiss newspapers.

The SSC for sheep had two main increases, whereby the first increase being due to the activity mentioned above, along with a second increase in DA brought by the education program destined for a selected number of sheep farmers and union members.

The overall performance along with the individual output sensitivities of the three SSCs are seen in Figure 9.1. Disregarding the sentinel surveillance system which was in place prior to 2007, the combined Se of the passive clinical surveillance both in sheep and cattle was considered as 46% until the month of June. At this time point, the Bulk Milk testing program was introduced and raised the combined Se to above 90%.

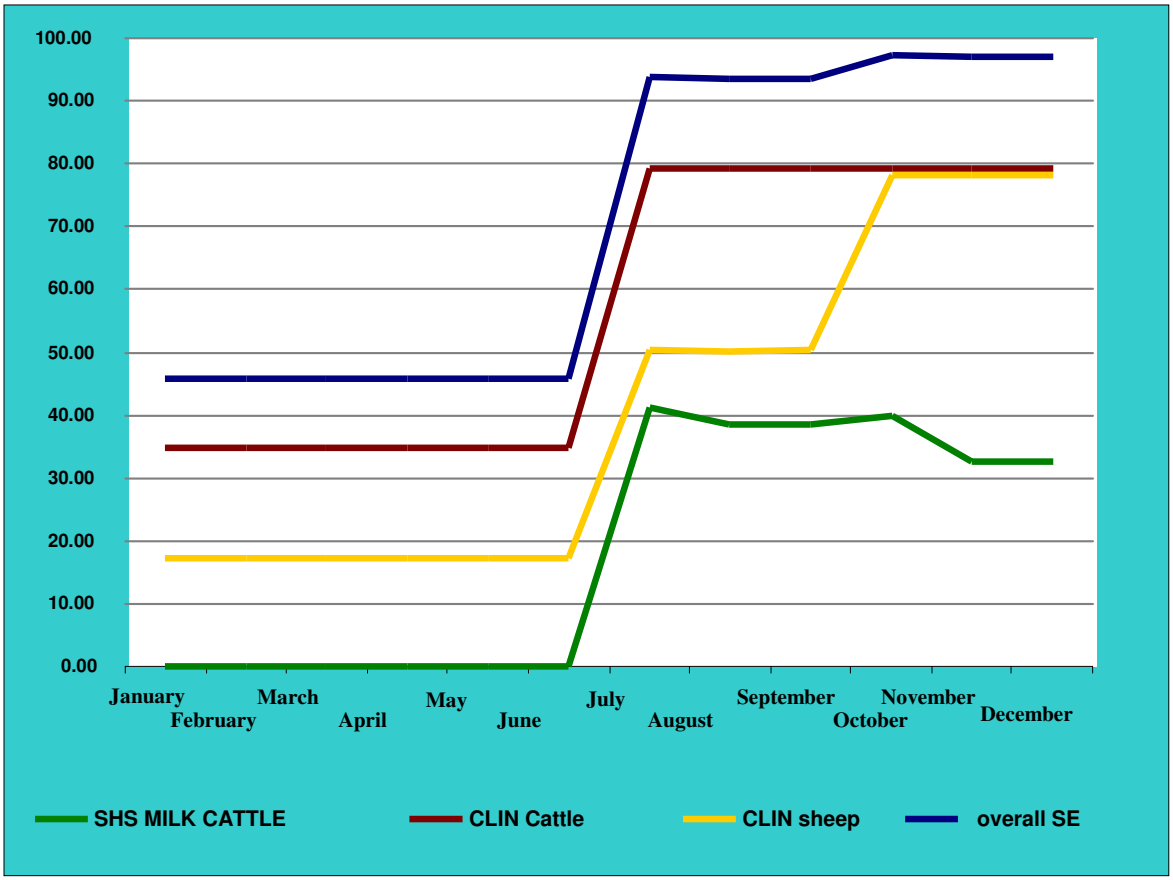

Figure 9.1: Overview of BT Scenario Tree sensitivity results for bluetongue surveillance in Switzerland.

DISCUSSION

The results of this scenario tree model enabled decision makers to establish a national surveillance plan concerning bluetongue disease. The model emphasized the importance of disease awareness levels and their effect on detection probabilities. Hence, various workshops and meetings were organized incorporating stakeholders and animals holders, especially sheep farmers. Direct results could be seen with the amount of clinical suspect cases being reported when compared to previous years. It also showed that the sentinel bulk milk testing SSC, although not as sensitive as a blood serological screening, allowed the overall Se of the program to reach adequate levels within financial limits. From July onwards, regular bulk milk testing was carried out which has also led to false positive findings. In these cases, serological blood testing of the whole herd involved was carried out. As mentioned, two recent bulk milk samples from the canton of Basel-Land and Vaud tested positive, and subsequent serological blood sampling resulted in two BT positive cattle in November 2007 and January 2008 respectively (www.bluetongue.ch).

The results highlight the importance of the quality of input parameters and their effect on the overall sensitivity of the surveillance system. The input parameters involved for disease awareness levels were very decisive in increasing the overall performance of the surveillance program. In previous models (data not shown), various simulations were carried out with either a combination of DA levels, or running the whole model on a single DA level. Therefore in order to create realistic circumstances, modest DA levels were used in the final simulation in order not to develop an over confident result. In the future it is planned to update the model with real data originating from various sources such as the level of media interest, numbers of suspect cases being reported, number of actual bulk milk testing samples, real data on veterinarian and farmer contacts and correct diagnostic procedures, as well as the amount of enquiries coming to the Federal Veterinary Office from the public concerning bluetongue disease.

The variations seen in terms of the Se on the sentinel bulk milk testing component emphasize the difficulty of creating a surveillance system concerning vector-borne diseases. Environmental and transhumance factors, along with climatic fluctuations will affect the activity and survival levels of the vector, and in turn alter the overall surveillance sensitivity by lowering or increasing the detection levels.

Each country uses different methods for BT surveillance depending on the current disease status, geographical barriers, allocated finances and resources available. The main difference between countries involved in the current northern European outbreak and Switzerland is the level of serological blood sampling strategy. Prior to BT outbreaks in Switzerland, this testing strategy increase the overall SE slightly, yet carried a substantial economical weight.

The output of the scenario tree model is as good as the quality of the input parameter information. The other important factor affecting the overall sensitivity of the scenario tree is the disease awareness levels. Switzerland detected its first BT case in late October through clinical surveillance. In the last six months, reporting of suspect cases has increased dramatically as compared to previous years (Dr. B. Thür, personal communication), which could be the consequences of an improved disease awareness level. In terms of input parameters based on entomological information, due to the localized data based on Swiss *Culicoides* activity, this might differ in geographically diverse regions.

In conclusion, through the analysis of various bluetongue disease surveillance options, three SSCs were chosen to create a national program for the early detection of BT in Switzerland. By identifying the most influential input parameters, actions were taken to strengthen these parts of the surveillance system. This resulted in the production of a BT documentary of which as many as 3000 copies have already been delivered. Additionally, workshops on the disease have been organized in different regions of the country in order to increase disease awareness levels, especially in the sheep industry. Therefore, through this scenario tree model, it was possible to create a risk based surveillance system on a national basis concerning an emerging disease through the incorporation of epidemiological and disease based information, and the ability to target weaker areas of disease awareness and information distribution.

As mentioned, this method could be improved by incorporating actual information on BT epidemiological data and disease awareness levels which is planned for the near future.

CHAPTER 10

The main objective of this thesis was to develop a sentinel herd surveillance program serving as an early warning system for vector-borne diseases, with main focus on bluetongue disease. The objectives were fulfilled through the following three paths as recommended by the EU (Anonymous, 2007) for a BT free zone, which suggests surveying areas in order to confirm absence of BT as well as to detect a primary incursion. This surveillance consists of i) serological/virological, ii) entomological surveillance and iii) optional clinical surveillance. A further recommendation is the collection of data, which includes geographical, meteorological epidemiological and entomological information specific for the area under observation, in order to determine risk of virus introduction either through infected hosts or infective vectors to enhance preventative measures.

Fulfilments of study objectives

Objective: ***Determining the immune status of the chosen sentinel herds in terms of the following three diseases: bluetongue disease, cattle anaplasmosis and cattle babesiosis***

Through the regular yearly serological blood sampling in areas considered at higher risk of BT occurrence based on vector biology as well as areas known to have tick presence, the sentinel herd program supported the absence of disease status for BT until the recent outbreak in October 2007. In the case of *Anaplasma marginale* and *Babesia divergens* it was more difficult to prove complete freedom due to the cross reactivity of the diagnostic tests used, which did not rule out the presence of another tick-borne infection present in the same areas for the duration of the surveillance. Based on the sentinel testing strategy, this alone would not have been adequate to prove disease freedom. Yet, due to the basic national survey on BT conducted in 2003 which

proved disease freedom (Cagienard *et al.*, 2006b), could continue to confirm its absence through its targeted risk based nature.

Objective: ***Study on abundance, distribution and population composition of BT vectors in Switzerland***

The entomological sampling part of the surveillance showed that a wide variety of *Culicoides* midges were present in relatively abundant terms throughout Switzerland. In 2004 though, BT was not believed to be transmitted in field conditions by certain *Culicoides*, such as some individual species belonging to the Obsoletus group. Yet through the duration of the thesis, it was shown that indeed, *Culicoides obsoletus* to name a few were in fact competent BT vectors as shown in Bulgaria (Purse *et al.*, 2006) and parts of Italy (Savini *et al.*, 2005) and discussed in Meiswinkels *et al.* manuscript describing the potential new *Culicoides* vector of BTV in northern Europe (Meiswinkel *et al.*, 2007). In Italy, BTV-2 and -9 were isolated from midges belonging to the *Obsoletus* complex, and in Bulgaria, an outbreak of BTV -9 occurred 1999 with the noted absence of the old world vector *C. imicola*. In this case, trapping samples were composed of 75% with individuals belonging to the *C. obsoletus* complex and could reach over 15,000 catch sizes per night, a figure similar as that found in Switzerland as shown in chapter 7 (Racloz *et al.*, submitted). Initial sentinel herds in 2004 were chosen according to risk areas of *Culicoides imicola* occurrence, and therefore were concentrated on the southern part of the country in the canton of Ticino. This was based on the single *C. imicola* species found in this canton, the warmer microclimate present, along with the presence of several BTV serotypes in Italy. During this study, as mentioned previously, the confirmation that other *Culicoides* species were able to transmit BT, and the creation of the risk maps highlighting northern parts of Switzerland suitable for vector presence prompted the establishment of further sentinel herds in the canton of Basel and Zurich, of which the former reported the first BT case.

Objective: ***Identification of areas considered at higher risk for disease incursion as well as BT vector establishment.***

This was achieved through the collection of climate, altitude and entomological data which served to create thematic maps highlighting a multitude of zones with varying risk levels.

The development and improvement of tools such as GIS, allowed the visualisation of areas at higher risk of disease occurrence and helping in decisions making processes. It also confirmed the importance of temperature and host availability on the dynamics of presence, frequency and population composition of the *Culicoides*.

The recent northern European outbreak of BTV-8 also inadvertently pushed forward an interest in this disease, and in doing so improved epidemiological and vector-based knowledge considerably. It also had the effect of emphasising the importance of a risk-based surveillance system, especially concerning vector-borne diseases. Since the species *Culicoides* is also involved in the transmission of many other important diseases, including African Horse Sickness, the current sentinel programme has the potential to serve as a good model to prepare for eventual incursion of these diseases into Europe.

Although this project focussed on the possible incursion mainly via an infected vector, other paths exist for the introduction of the disease into a BT-free zone/country. These would ideally be incorporated into the risk identification system. In general, illegal import of infected hosts, infected vector introduction via wind (as is thought to be the case for the United Kingdom and Switzerland concerning BTV-8), plants (possible path via flower export industry from Africa to the Netherlands), transport vehicles or fomites, as well as the use of semen or embryo and vaccine material are all possible introduction routes. Even legal import have some risk as shown in the recent case of BTV-8 in Italy in the Parma region, which is believed to have occurred through the import of cattle originating from France (www.promedmail.org) This might have serious implications due to the presence of *C. imicola* in Italy along with several other BTV serotypes (Anonymous, 2007). These other risk paths have been under scrutiny in the northern European outbreak of BTV-8 where as of date, no concrete cause has been found, although through phylogenetic analysis it was closely linked to the strain found in Nigeria in 1982. Nevertheless, it is important to mention that BTV-8 has also been detected in certain areas of Africa, Pakistan, India, Central and Southern America as well as recently in Europe (www.iah.bbsrc.ac.uk). In terms of the cases in Switzerland, these occurred at the end of October and it is highly presumable that they originated from infected vectors coming into the northern parts of Switzerland in the cantons of Basel and Solothurn, from Germany.

In terms of risks of the introduction of *Anaplamsa marginale* and *Babesia divergens*, due to the lack of epidemiological knowledge on the distribution of these specific agents, resources were concentrated on determining habitat areas of their main European vector, *Ixodes ricinus*. Similar to *Culicoides* vectors, these are affected by a number of environmental factors with humidity, altitude and forest cover being most important (Estrada-Peña, 2006). Figure 10.1 shows general suitability maps for the vector *Ixodes ricinus* for 2006 in Switzerland, based on temperature, humidity, altitude and forest cover.

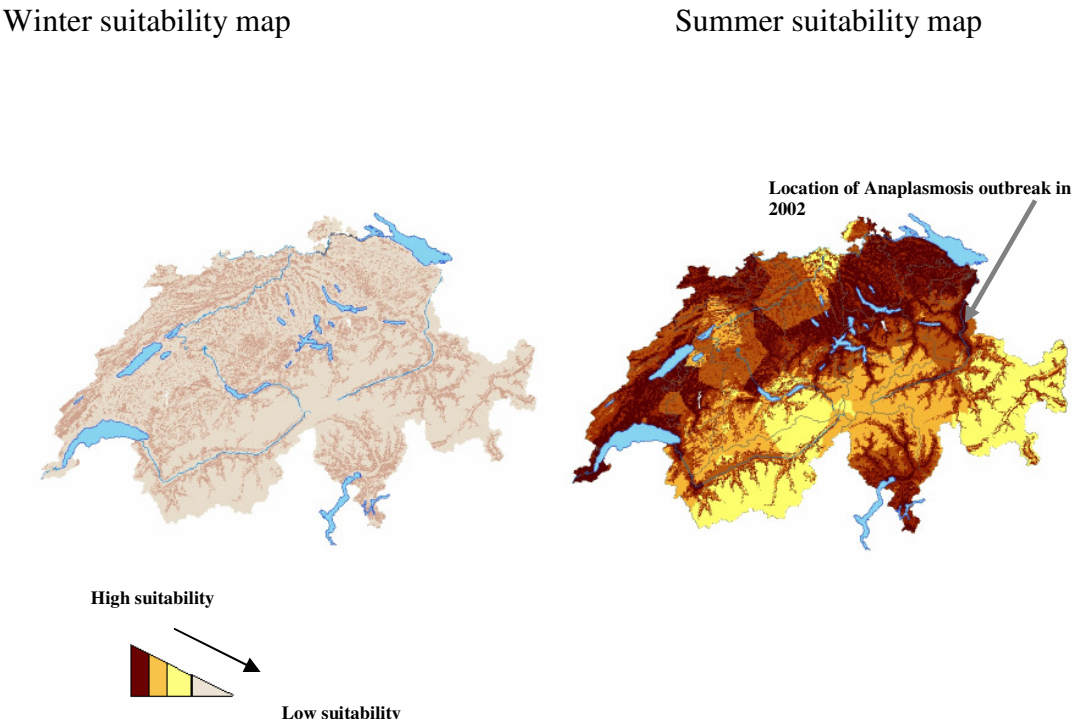

Figure 10.1: Suitability maps for winter and summer 2006 for the habitat of *Ixodes ricinus*, highlighting areas of low to high risk for disease transmission (unpublished data).

As seen in Figure 10.1, similar areas were considered of higher risk for disease transmission as the maps created for BT disease (Figure 6.4). This is due to the high dependence on altitude levels. Therefore certain Alp and pre-Alp areas were always considered low risk for disease occurrence via infected vectors.

Limitations of study

The ability to create an early warning system through the combination of climate, environmental, host and vector based data through the visual aid of GIS can be used in many ways to improve veterinary and public health surveillance systems. GIS has also been used as a data recording tool, to model disease spread, to help decision makers in disease control as well as allowing statistical assessments to be carried out through cluster analysis. Yet, although much improved in the last decade, the use of GIS still has its limitations depending on the purpose of the work.

In this study, the accuracy of the maps could have been improved in chapter 8 by incorporating wind movement based on the assumption that a primary incursion would occur through an infected vector. Another limitation as mentioned is the occurrence of importing an infected cattle, and due to the mixed clinical symptom levels in the current years outbreak, the potential for it to be moved to an area considered low risk was possible. At this location, although vector presence is low, there could have been transmission or 'quiet' spread of disease.

Another limitation to this study is the application or feasibility of the surveillance system components recommended by the outcome of the scenario tree. In reality, although the number of sentinel herds used in the scenario tree model was 200, the real data has shown that due to administrative and practical reasons, only circa 170 herds are regularly being tested for BT. When running these figures in the model, it brings down the overall sensitivity level by a considerable amount. Yet to counter balance, DA levels used were on the modest side, and from current data could be increased.

The use of remote sensing and satellite-derived environmental variables could also improve the use of GIS as an early warning system, or as a predictive tool (Kalluri *et al.*, 2007). As soon as more information is available on the exact biology of the vectors involved, maps could be produced including parameters such as land cover type, soil composition along with multi-temporal satellite data.

Application of results from this study

As discussed in Chapter 9, data deriving from the entomological sampling as well as those for climate and altitude parameters were used to create thematic maps in order to help visualise

areas considered at higher risk for BT occurrence based on vector biology. Finally, through the creation of monthly suitability maps which highlighted the change in risk levels depending on climate and vector activity levels, a scenario tree model was created to expand the previous sentinel surveillance program into a targeted, national program. This was composed of serological sampling of 200 dairy herds for presence of BT, as well as increasing disease awareness programs to aid in clinical surveillance of cattle and sheep. Figure 10.2 shows the location of the new sentinel herds among the location of all cattle farms in Switzerland. The background colour indicates general level of risk ranging from low to high, whilst the red circles indicate the areas of Swiss BT outbreaks.

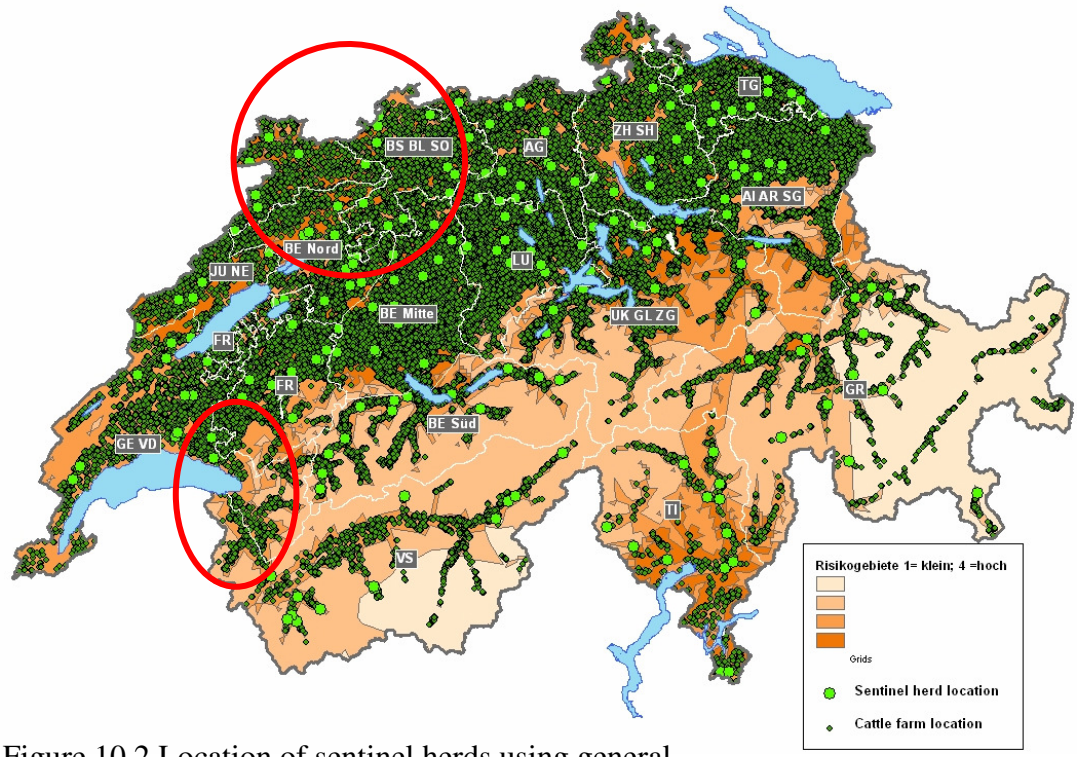

Figure 10.2 Location of sentinel herds using general suitability map. Location of first incursion of BT circled in red.

As of date, a regular bulk milk testing program is running on a monthly basis covering circa 170 dairy farms located in areas considered of heightened risk for BT occurrence based on vector biology. Several bulk milk batches had tested false positive since the program began in July, and confirmatory sampling sessions were carried out by targeting all cattle on the affected farm through individual serological blood sampling. Recently the bulk milk testing strategy detected two cases of BTV-8 in the cantons of Basel and Valais. The other important factor

affecting the overall sensitivity of the scenario tree is the disease awareness levels. In the last six months, reporting of suspect cases has increased dramatically as compared to previous years (B. Thür, personal communication), which could be the consequences of an improved disease awareness level. This will be measured in a further study.

Implications of the recent BT cases in Switzerland

As seen in Figure 10.2, and Figure 6.4, the seven farms affected by BT were located in areas considered at high risk of BT occurrence based on vector biology. Five of these outbreaks were identified through clinical suspect cases, whilst two were detected in the bulk milk testing program. Out of a total of 609 susceptible cattle from the farms located in Basel-Stadt, Solothurn, Basel-Land, Valais and Jura, it was detected in 12 cattle (and 2 goats), with prevalence rates ranging from 0.63% to 10.3%. The number of affected cattle on each farm relate to the predicted basic reproductive numbers for that region calculated for 2006, as seen in Figure 8.1 and the maps provided in the annex. Interestingly to date, the method and rate of detection correspond to the predicted SSC performances mentioned in chapter 9.

Although the maps now would not be termed as an early warning system for primary incursion of BT or infected vectors any longer, they still serve the purpose of identifying areas where possible higher concentrations of the disease could occur, given that updated maps are created as soon as possible with recent climate data. With current legislation concerning movement restrictions in place, the maps still help in the visualization of areas where DA levels should be at highest due to *Culicoides* presence. Yet if trading, mixing of cattle on alpine pastures or livestock events from different areas occur, since BT virus is present in the country, the maps would be less accurate in predicting BT prone areas based on vector presence alone.

In the case of Europe, where BT has been spreading quite rapidly in the last decade, early warning systems might be useful to monitor the different serotypes, yet once endemic, or the use of vaccination has been decided upon, it would no longer serve the purpose of detecting early disease incursion. In other countries, such as Australia where there is a more consistent geographical limit of disease occurrence, the sentinel surveillance program in place is an important strategy since BT has not affected highly dense areas of sheep livestock further south from the sentinel herds.

Since certain BT vectors are also transmitters of other diseases such as African Horse Sickness, it is still a valid approach to detect these diseases in currently free zones/countries. In terms of *Culicoides* surveillance, gaps in the knowledge of vector biology and life cycle, as well as the relation to climate and environmental factors remain. It is also needed in EU regulations to allow trade to recommence in affected areas as soon as the 'vector free' period is declared.

Conclusions

The main objective of the thesis was to create an early warning system for the establishment of vector-borne disease in Switzerland. At this point, BT was occurring mainly in the Mediterranean area, and involved many different BTV serotypes, yet not BTV-8. The initial focus of the surveillance program was in the southern parts of Switzerland due to the climate and the proximity to both BT and *C. imicola* in Italy. Once the thematic maps were created, it showed that other parts of Switzerland in particular the northern plateau were also at higher risk of BT occurrence due to the shift in vector species that the new outbreaks in the Balkan areas were caused by. This prompted the expansion of the sentinel herds into other parts of Switzerland in terms of serological and entomological surveillance. Recent BTV-8 outbreaks in Northern Europe in 2006 finally spread into Switzerland into an area considered at high risk of BT occurrence by October 2007. There still remains a considerable threat for the southern part of Switzerland in terms of both the establishment of *C. imicola* in the next few years, as well as the incursion of different BTV serotypes.

In conclusion, this study emphasized the usefulness of using up to date technology and epidemiological knowledge to aid in the surveillance of disease in general. It has also highlighted the difficulty in monitoring vector-borne diseases due to the multitude of entry paths possible, yet hopefully served as the basis for improving disease awareness, possible alternative applications and surveillance methods as well as interest levels. This model can therefore serve as a tool concerning vector-borne disease of veterinary relevance, as well as forming the model basis of important human zoonotic vectors which are likely to come into Europe, and in particular Switzerland.

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Languages: English (mother tongue), French (fluent), German & Spanish (basic)

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Educational Experience

Swiss Federal Veterinary Office, Bern, CH : 2004-2007
PhD on vector borne disease surveillance in Switzerland (University of Basel)

Royal Veterinary College, London, UK 2003-2004
MSc (distinction) in Control of Infectious Disease in Animals

University of London, Royal Holloway, UK 1998-2002
BSc (honours) in Zoology

Ecole Moser, Geneva, CH: 1999-2000
Swiss Federal Baccalaureate degree (economics major)

Southbank International, London, UK 1996-1998
International Baccalaureate degree (science major)

American Community School, Cobham, UK 1994-1996
GCSE exam in Japanese (Grade A)

International School of the Sacred Heart, Tokyo, Japan 1986-1994

Work Experience

Swiss Tropical Institute, Basel, CH Jan. 2008- at present
- Postdoc position on global epidemiology of bacterial meningitis

Veterinary Laboratory Agency, Weybridge, UK April – July 2004
- Study of Arctic rabies in Greenland
- RT-PCR, sequence analysis, phyolgenetics
- Supervised by Dr. Tony Fooks, virology department

World Health Organisation Vaccinology Centre, CH Oct 2002-Aug 2003
- University of Geneva Medical Centre
- Involved in learning immunological techniques (ELISA, ELISPOT, LDA, CTL chrome release assay and in vitro restimulation using murine models)

- Specifically analysing the T cell (CD4 and CD8) response after immunisation with a live attenuated Measles virus
- Supervised by Dr. Claire-Anne Siegrist

World Health Organisation, Advanced Vaccinology course, CH Summer 2002

University of Lausanne and Fondation Merieux, Annecy, France

- Participated as an observer

World Health Organisation, Geneva Headquarters, CH Summer 2001

- Internship in Communicable Disease Cluster, supervised by Dr Max. Hardiman, Communicable Diseases Department
- Involved in collecting and analysing data on meningococcal disease in sub-Saharan Africa for season of 2001
- Produced report for the Weekly Epidemiological Record

Additional Information

- Born in South Africa and lived in Venezuela, Japan, England and Switzerland
- Enjoy travelling and learning about different cultures
- Member of SCS, Bern volleyball team, also a keen swimmer and horse rider

Referees

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Publications:

Submitted:

S. Casati & V. Racloz, J.C. Delécolle, M. Kuhn, A. Mathis, C. Griot, K. D.C. Stärk and T. Vanzetti. **Entomological monitoring of bluetongue vectors at sentinel sites in southern Switzerland: an investigation on the *Culicoides* population composition** (Medical and Veterinary Entomology)

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V. Racloz, H. Schwermer, C. Griot, K.D.C. Stärk and Hadorn D.C **Establishing a national surveillance system for bluetongue in Switzerland using Scenario Tree modelling** (Trounsboundary Emerging Diseases)

Published:

Racloz V, Presi P, Vounatsou P, Schwermer H, Casati S, Vanzetti T, Griot C, Stärk KD 2007. **Use of GIS maps and statistical modeling for the prediction of Bluetongue disease occurence in Switzerland based on vector biology.** Veterinaria Italiana. 43 (3)

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Racloz V, Hardiman M, 2001. **Epidemics of meningococcal disease, African meningitis belt.** WHO Weekly Epidemiology Record (September 2001)

Conferences, workshops and courses attended during PhD:

Conferences

TTP5, University Neuchatel, Aug 28th – Sep 2nd 2005

- 5th International Conference on Ticks and Tick-borne Pathogens

Buiatrissima conference, Bern , Oct. 20 - 21st 2005

- Establishment of an early warning system for vector-borne diseases in Switzerland *poster*

ISVEE XI, Cairns, Australia, Aug. 6 – 11th 2006

- International Symposium on Veterinary Epidemiology and Economics
- Sentinel Surveillance Systems with Special Focus on Vector-Borne Diseases *presentation*

DVG, University Bern, Sep. 6 – 8th 2006

- Schweizer Forum für Epidemiologie und Tiergesundheit
- Determining the efficacy of a risk-based surveillance of Bluetongue disease in Switzerland *poster*

1st OIE International Conference, Pescara, Italy Oct. 8 – 11th 2006

- Use of GIS in Veterinary Activities
- Use of GIS maps and statistical modeling for the prediction of Bluetongue disease occurrence in Switzerland based on vector biology *poster*

Medreonet , CIRAD Montpellier, France, Jan. 31st – Feb 2nd 2007

- Surveillance network of reoviruses, Bluetongue and African Horse Sickness in the Mediterranean Basin and Europe
- Summary of Bluetongue entomology surveillance in Switzerland *presentation*

GISVET 2007, 20-24th August, Denmark

- Demonstrating changes in disease risk status for vector-borne diseases : Use of GIS and statistical modeling *presentation*

SVEPM, Liverpool UK, March 26th-28th 2008

- Society for Veterinary Epidemiology and Preventive Medicine
- *Presentation* on BT from research to policy.

Workshops, courses

NOSOVE GIS workshop, Hässelby Slott**,** Sweden, Jan. 18 – 21st 2005

- Nordic Society for Veterinary Epidemiology

Animal Experimentation certificate, Zurich University, July 4 – 8th 2005

Epidemiology and Biostatistics course, Bern, Aug. 8 – 19th 2005
- Uni. Bern Vetsuisse course

Vector-borne exotic disease workshop, London, Oct. 27 - 28th 2005
- Society of Chemical Industry, Pest Management Science

Scientific writing course, April 21st - May 5th 2006
- University Bern

Other

University of Bern: (January + November 2005, 2006)
- *Presentation* on Bluetongue disease surveillance in Switzerland

BVET – Environmental Systems Research Institute (ESRI) meeting
- *Presentation* on use of GIS in BT surveillance

Ceratopogonidae Information Exchange (CIE) newsletter, May 2007:
- Summary of Bluetongue entomology surveillance in Switzerland. *letter*

BVET Magazine Volume 1, 2007
- Anaplasmosis, Babesiosis and Theileriosis. *article*

University of Zurich, September 25th 2007
- Bluetongue vectors in Switzerland *presentation*

Royal Veterinary College London and BVET Bern, August and October 2007
- Workshop on Scenario tree models *presentation*